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Contents

Cancer Treatment Reports
Volume 66, Number 7
July 1982

Page

ARTICLES

Current Controversies

The Role of Intensive Postinduction Chemotherapy in the Management of Patients With Acute Myelogenous Leukemia 1455
Robert J. Mayer, Howard J. Weinstein, Felice S. Coral, David S. Rosenthal, and Emil Frei, III
Sidney Farber Cancer Institute, Boston, Massachusetts

Marrow Transplantation for Patients With Acute Nonlymphoblastic Leukemia Who Achieve a First Remission 1463
E. Donnall Thomas, Reginald A. Clift, and C. Dean Buckner
Seattle Marrow Transplant Team

Therapy for Patients With Acute Myelocytic Leukemia Who Enter Remission: Bone Marrow Transplantation or Chemotherapy? 1467
H. D. Preisler
Roswell Park Memorial Institute, Buffalo, New York

Clinical Studies

AMSA—A Promising New Agent in Refractory Acute Leukemia 1475
H. Jeffrey Lawrence, Curt A. Ries, Ralph D. Reynolds, Jerry P. Lewis, Maria M. Koretz, and Frank M. Torti
University of California-Davis, Sacramento

Clinical Evaluation of Succinylated *Acinetobacter* Glutaminase-Asparaginase in Adult Leukemia 1479
Raymond P. Warrell, Jr, Zalmen A. Arlin, Timothy S. Gee, Ting-Chao Chou, Joseph Roberts, and Charles W. Young
Memorial Sloan-Kettering Cancer Center, New York, New York

The Course of Nausea and Vomiting After High-Dose Cyclophosphamide 1487
John H. Fetting, Louise B. Grochow, Marshal F. Folstein, David S. Ettinger, and Michael Colvin
The Johns Hopkins University School of Medicine, Baltimore, Maryland

Page

Essential Thrombocythemia: Response During First Year of Therapy With Melphalan and Radioactive Phosphorus: A Polycythemia Vera Study Group Report 1495
Scott Murphy, David S. Rosenthal, Aleksander Weinfeld, Jean Briere, Guy B. Faguet, William H. Knospe, Stephen A. Landaw, John Laszlo, Anthony V. Pisciotta, Anthony P. Tartaglia, Judith D. Goldberg, Paul D. Berk, Paul B. Donovan, and Louis R. Wasserman
Polycythemia Vera Study Group

Management of Pelvic Complications of Malignant Urothelial Tumors With Combined Intra-Arterial and Iv Chemotherapy 1501
Christopher J. Logothetis, Melvin L. Samuels, Sidney Wallace, Vincent Chuang, Antonio Trindade, Cynthia Grant, Thomas P. Haynie, III, and Douglas E. Johnson
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Phase I Study of Indicine *N*-Oxide in Patients With Advanced Cancer 1509
Takao Ohnuma, Kasi S. Sridhar, Lynn H. Ratner, and James F. Holland
Mount Sinai School of Medicine, New York, New York

Phase I Clinical Evaluation of 9,10-Anthracenedicarboxyaldehyde[bis(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone]dihydrochloride (Bisantrene) 1517
Boh-Seng Yap, Hwee-Yong Yap, George R. Blumenschein, Agop Y. Bedikian, Robert Pocelinko, and Gerald P. Bodey
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Preclinical Pharmacology

Cyclophosphamide-Induced Cardiomyopathy in the Rat 1521
Harold A. Hopkins, William L. Betsill, Jr, Ann S. Hobson, and William B. Looney
University of Virginia School of Medicine, Charlottesville

Experimental Ultrastructural and X-ray Microanalysis Study of Cisplatin in the Rat: Intracellular Localization of Platinum 1529
J. P. Berry, P. Brille, A. F. LeRoy, Y. Gouveia, P. Ribaud, P. Galle, and G. Mathé
Faculté de Médecine, Créteil, France

Development and Characterization of a Cyclophosphamide-Resistant Mouse Plasmacytoma Cell Line 1535
Carmelita G. Frondoza, Sudhir M. Trivedi, and Richard L. Humphrey
The Johns Hopkins University School of Medicine, Baltimore, Maryland

Modification of Radiation Damage in Rat Spinal Cord by Mitotane 1545
Arvin S. Glicksman, Sarah F. Bliven, and John T. Leith
Brown University, Providence, Rhode Island

BRIEF REPORTS

Treatment of Neoplastic Meningitis With Intraventricular Thiotepa and Methotrexate — 1549
Donald L. Trump, Stuart A. Grossman, George Thompson, Kenneth Murray, and Moody Wharam
Johns Hopkins Medical Institutions, Baltimore, Maryland

Sequential Methotrexate and 5-FU in the Treatment of Colorectal Cancer — 1553
B. Weinerman, B. Schacter, H. Schipper, D. Bowman, and M. Levitt
University of Manitoba, Winnipeg, Canada

CCNU, Vincristine, Methotrexate, and Procarbazine Treatment of Relapsed Small Cell Lung Carcinoma — 1557
Elizabeth A. Poplin, Joseph Aisner, David A. Van Echo, Margaret Whitacre, and Peter H. Wiernik
University of Maryland Cancer Center, Baltimore

Neurologic and Cardiac Toxicity Following Iv Application of Methotrexate — 1561
Adrian B. Gasser, Marcell Tièche, and Kurt W. Brunner
Institute of Medical Oncology, University of Bern, Inselspital, Switzerland

Phase II Study of Sequential Methotrexate-5-FU Therapy in Advanced Measurable Colorectal Cancer — 1563
James E. Cantrell, Jr, Rene Brunet, Claude Lagarde, Philip S. Schein, and Frederick P. Smith
Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington, DC

Phase II Trial of Doxorubicin Therapy for Advanced Islet Cell Carcinoma — 1567
Charles G. Moertel, Philip T. Lavin, and Richard G. Hahn
Eastern Cooperative Oncology Group

In Vitro Evaluation of the Cardiotoxic Potential of AMSA — 1571
Michael C. Lowe
National Cancer Institute, Bethesda, Maryland

Effects of Interferon on C1300 Mouse Neuroblastoma — 1575
Mutsuhiko Tozawa, Takuro Kidowaki, Terufusa Tanaka, Tadashi Sawada, Tomoichi Kusunoki, Tohru Oku, and Tsunataro Kishida
Kyoto Prefectural University of Medicine, Japan

Parenteral Formulation of Hexamethylmelamine Potentially Suitable for Use in Man — 1579
Matthew M. Ames and John S. Kovach
Mayo Clinic, Rochester, Minnesota

CLINICAL TRIALS SUMMARIES

Page

Phase II Trial of Prednimustine in Children With Recurrent Cancer: A Children's Cancer Study Group Report 1583
Paul S. Gaynon, Edward S. Baum, William Krivit, and Denman Hammond
Children's Cancer Study Group

Phase II Trial of Combination Methyl-GAG and Vinblastine in the Treatment of Metastatic Renal Adenocarcinoma 1585
Robert F. Todd, III, Marc B. Garnick, and George P. Canellos
Sidney Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts

Phase II Evaluation of Maytansine in Refractory Non-Hodgkin's Lymphoma: A Southwest Oncology Group Study 1587
Voravit Ratanatharathorn, Nazli Gad-el-Mawla, Henry E. Wilson, John D. Bonnet, Saul E. Rivkin, and Robert Mass
Southwest Oncology Group

Phase II Trial of AMSA in Previously Treated Patients With Stage III and IV Ovarian Cancer 1589
R. J. Schneider, T. M. Woodcock, J. Howerd, and M. Ochoa, Jr
Memorial Sloan-Kettering Cancer Center, New York, NY

Phase II Evaluation of Gallium Nitrate in Breast Cancer: A Southwest Oncology Group Study 1591
Carol J. Fabian, Laurence H. Baker, Clarence B. Vaughn, and Harry E. Hynes
Southwest Oncology Group

LETTERS

Case Report: Acute Leukemia After Semustine (Methyl-CCNU) Therapy 1593
William J. Uphouse and Noboru Oishi
University of Hawaii at Manoa, Honolulu

Experience of the Eastern Cooperative Oncology Group With Doxorubicin as a Single Agent in Patients With Previously Untreated Breast Cancer 1594
Samuel G. Taylor, IV, and Richard D. Gelber
Eastern Cooperative Oncology Group

Pharmacology and Toxicity of Ip Streptozocin in Ovarian Cancer: A Case Report 1595
Lawrence C. Panasci, Violetta Skalski, Judith St-Germain, Philip Lazarus, Michael Shinder, and Richard Margolese
McGill Cancer Center, Jewish General Hospital, Montreal, Quebec, Canada

Trial of AMSA in Acute Leukemia 1596
Juan C. Dupont, Guy E. Garay, Cristina Scaglione, and Santiago Pavlovsky, and Paul V. Woolley
Academia Nacional de Medicina, Buenos Aires, Argentina

ANNOUNCEMENTS

	Page
Approaches to the Management of Pain	1599
Urologic Cancer Course	
Paraneoplasia: Basic Concepts and Clinical Aspects	
Gastrointestinal Tumor Study Group	

The Role of Intensive Postinduction Chemotherapy in the Management of Patients With Acute Myelogenous Leukemia[1]

Robert J. Mayer,* Howard J. Weinstein, Felice S. Coral, David S. Rosenthal, and Emil Frei, III [2]

During the past decade, combination anthracycline and cytarabine chemotherapy, sometimes accompanied by 6-thioguanine, has been associated with an increase in the complete response rate from 35%–55% (1,2) to approximately 75% (3–6) in patients < 60 years old having acute myelogenous leukemia (AML). This encouraging advance, however, has not been associated with prolonged periods of remission and indefinite survival in a significant number of patients, as have been seen in children with acute lymphocytic leukemia (ALL). Despite the use of "maintenance" chemotherapy, the median duration of remission in most recent investigations of AML is 12–15 months (3–14), with the long-term survivorship among such patients being 20%–25% (2,12,14,15). The frustrations encountered in attempting to improve these results have led some investigators to question the appropriateness of aggressive antileukemic therapy in terms of the human suffering caused by such treatment (16), thereby implying that AML is a basically incurable disorder.

Before accepting the inevitability of relapse in the patient with AML, it seems wise to reassess the meaning of maintenance therapy in this disease and to determine whether past therapeutic efforts have adequately addressed the problem of eradicating clinically undetectable residual blast cells during remission.

Maintenance Therapy in Childhood ALL

On the basis of postmortem analysis and cell-cycle time determinations, the leukemic cell burden in a child with ALL has been estimated to be in the range of 1.0×10^{12} cells (17). Treatment with chemotherapy leading to the achievement of a complete remission is thought to result in a 1000–10,000-fold reduction in the number of leukemic cells (17). The discontinuation of drug treatment immediately after remission induction leaves the patient with the clinically undetectable presence of 10^8–10^9 leukemic cells, making subsequent relapse a near certainty. Clinical experience has offered support for this concept. A prospective trial performed 20 years ago in which children with ALL in remission were randomized to 6-mercaptopurine maintenance therapy or a placebo clearly demonstrated that additional drug treatment leads to an increased duration of remission (18). The introduction of maintenance therapy, particularly drug combinations, to further reduce and eliminate residual leukemic cells has been one of the fundamental steps leading to the long-term disease-free state presently attained by most children with this disease (19).

The acute toxicity when children are treated for ALL is far milder than that observed with therapy for AML. Complete remission in childhood ALL can generally be achieved without the development of marrow aplasia. Lymphoblasts are extremely sensitive to relatively nonmyelosuppressive agents (eg, vincristine, corticosteroids, and L-asparaginase) as well as to drugs that damage bone marrow elements (eg, methotrexate, 6-mercaptopurine, and anthracyclines). Since the chemotherapeutic agents employed in the treatment of childhood ALL have been associated with "acceptable" side effects and a gratifying therapeutic outcome, there has been no reluctance to continue the use of maintenance therapy in this disease.

Should the Therapeutic Principles Utilized in ALL be Applied to AML?

Whereas ALL appears to be a neoplasm that has infiltrated the bone marrow, AML represents a cytogenetically definable clonal expansion (20,21) of abnormal hematopoietic cells having a maturation defect at one of several possible sites in the differentiation pathway emanating from the pluripotent stem cell (22). In contrast to the situation in ALL, leukemic myeloblasts are only moderately sensitive to chemotherapy. Complete remissions in AML can usually be achieved only through the use of inten-

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sive drug treatment leading to bone marrow aplasia. Therapeutic strategy is based on the premise that normal marrow elements will recover more rapidly than abnormal, leukemic clones. The recent improvement in the remission-induction rate in patients with AML (3–6) is a reflection of higher, more intensive doses of chemotherapy as well as the added availability of broader-spectrum antibiotics and platelets for transfusion to "support" such individuals through periods of extreme myelosuppression.

There is every reason to believe that patients with AML in complete remission harbor a similar leukemic cell burden (ie, 10^8–10^9 cells) as has been estimated for children with ALL (17). In keeping with the experience in ALL, patients with AML in complete remission may be expected to relapse within several months if no further treatment is administered. It would furthermore seem reasonable that continued (ie, maintenance) chemotherapy of the type that led to complete remission should be effective in extending the period of remission by further decreasing the number of residual leukemic cells. Such treatment would seem essential if the goal in the treatment of patients with AML is not palliation but cure.

Does Maintenance Therapy Prolong Remission in AML?

It is difficult to compare published data reporting the duration of remission in patients with AML. Most studies are limited by their small patient size and short followup times. Perhaps the greatest impediment to such comparisons is the variety of remission-induction regimens utilized prior to patient evaluation during the postremission period. Drug treatment plans associated with a high remission-induction rate may quite possibly be more intensive, thereby leading to a greater "leukemic cell kill," resulting in a longer period of remission without maintenance therapy. In contrast, somewhat less intensive induction regimens followed by relatively low-dose maintenance plans may well lead to a total remission duration equal to that achieved by quite intensive induction programs alone.

The experience accumulated in seven series (8,9,11,13,23–25) in which AML patients in complete remission were given no maintenance therapy is outlined in table 1. Four of these patient groups (8,9,11,13) received several courses of "consolidation" treatment after remission was achieved, while the other three (23–25) were given no postinduction therapy. The complete remission rate in these clinical trials ranged from 40% (23) to 60% (13), reflecting in large part the intensity of the induction regimen. The median duration of remission for these unmaintained patients varied from 2.0 months (23) to 9.5 months (25), again a measure of the dose and schedule of the induction treatment. Regardless of the induction plan utilized, > 50% of these unmaintained patients relapsed within 10 months. A 1-year remission duration > 35% was reported in only one study (25). This clinical trial, performed by investigators at Johns Hopkins University, explored the effect of timed-sequential chemotherapy and was so intensive that marrow recovery in responding patients was not observed until almost 4 weeks after the start of treatment (25). While the probability of achieving a second remission may be increased in unmaintained patients (25), presumably through a reduction in the development of drug resistance,

TABLE 1.—Comparative published results: remission duration without maintenance therapy

Therapy*		Complete remission (%)	No. of patients in remission without chemotherapy	Median duration of complete remission (mos)	% in remission at 1 yr	Ref No.
Remission induction	Consolidation					
DNR or POMP	None	40	17	2.0	12	23
Ara-C, 6-TG	Ara-C, 6-TG (3 mos)	41	24	5.3	10	9
DNR, Ara-C, 6-TG†	DNR, Ara-C, 6-TG (2 mos)	51	37‡	6.7	Not stated	11
DNR, Ara-C, 6-TG†	Ara-C, 6-TG (1 mo)	Not stated	13	6.7	0	8
DNR, Ara-C, 6-TG, ADR, Cyclo	ADR, Cyclo	60	15§	8.0	33	13
Cyclo, Ara-C, VCR	None	46	12	8.5	25	24
DNR, Ara-C	None	56	19	9.5	47	25

*DNR = daunorubicin; POMP = prednisone, vincristine, methotrexate, and 6-mercaptopurine; Ara-C = cytarabine; 6-TG = 6-thioguanine; ADR = doxorubicin; Cyclo = cyclophosphamide; VCR = vincristine.

†Part of randomized study vs maintenance chemotherapy.

‡18 of these patients received sc injections of BCG.

§Received BCG + irradiated allogeneic AML cells.

second remissions in nontransplanted patients with AML are of short duration and survival generally parallels the duration of the first remission.

The results of 11 representative studies (2,3,8–13,26–28) in which adults and children with AML in complete remission were given maintenance chemotherapy are detailed in table 2. In contrast to the observation in the unmaintained groups, nine of the ten maintenance trials in which sufficient data are available report that 35% of the patients have remained in continuous remission after 1 year. The four treatment plans having the highest remission-induction rates are associated with the longest median remission durations (> 12 months) and percentages of patients remaining in complete remission after 12 months (> 50%) (3,12,27,28). The maintenance programs in these four studies varied in intensity.

The data from tables 1 and 2 strongly suggest that the use of maintenance chemotherapy will prolong remission duration in patients with AML. This conclusion is supported by a prospective randomized study (8) in which five of 13 (38%) patients receiving modest doses of cytarabine and 6-thioguanine remained in continuous complete remission after 12 months, while none of 13 similar unmaintained patients remained disease-free. A second prospectively randomized study failed to show an advantage for the use of a relatively inactive drug (carmustine [BCNU]) in combination with a single monthly injection of cytarabine in the maintenance arm (11).

TABLE 2.—Comparative published results: remission duration with maintenance therapy

Therapy*			Complete remission (%)	No. of patients in remission	Median duration of complete remission (mos)	% in remission at 1 yr	Ref No.
Remission induction	Consolidation	Maintenance					
Ara-C, 6-TG	Ara-C, 6-TG (3 mos)	DNR, 6-TG, Cyclo, VCR, BCNU, hydrea (L-6 regimen)	41	24	6.0	39	9
DNR, Ara-C, 6-TG†	DNR, Ara-C, 6-TG (2 mos)	Ara-C, BCNU	51	17	7.0	Not stated	11
DNR, Ara-C, 6-TG, ADR, Cyclo	ADR, Cyclo	DNR, Ara-C, 6-TG	60	17‡	8.0	33	13
ADR, Ara-C, VCR, Pred	ADR, Ara-C, VCR, Pred	DNR, Ara-C, 6-TG	45	39§	8.0	42	10
Ara-C, 6-TG	Ara-C, 6-TG	DNR, 6-TG, Cyclo, VCR, BCNU, hydrea, MTX (L-6 regimen)	56	49	10.0	45	2
DNR, Ara-C, 6-TG†	Ara-C, 6-TG (1 mo)	Ara-C, 6-TG (monthly)	Not stated	13	10.3	38	8
Ara-C, 6-TG, Cyclo, Pred, VCR	None	Ara-C, 6-TG, Cyclo, VCR	59	94	11.5	48	26
DNR, Ara-C, 6-TG	DNR, Ara-C, 6-TG	DNR, Ara-C, 6-TG (24-mo duration)	82	56	13.0	55	3
DNR, Ara-C or DNR, Pred or Ara-C, 6-TG	None	Ara-C, 6-TG (60-mo duration)	58	26	17.0	52	12
ADR, Ara-C, VCR, Pred	None	ADR, Ara-C, azacitidine, MTX, 6-MP, Pred, VCR (VAPA-10 regimen—16-mo duration)	70	58	23.0	67	27
ADR or DNR, Ara-C	ADR, Ara-C, Cyclo (3 mos)	ADR, Ara-C, 6-TG, Cyclo, methyl-GAG (970701 regimen)	90	20	37.0	69	28

*Ara-C = cytarabine; 6-TG = 6-thioguanine; DNR = daunorubicin; Cyclo = cyclophosphamide; VCR = vincristine; BCNU = carmustine; hydrea = hydroxyurea; ADR = doxorubicin; Pred = prednisolone; MTX = methotrexate; 6-MP = 6-mercaptopurine.

†Part of randomized study vs no maintenance therapy.

‡Also received BCG + irradiated allogeneic AML cells.

§Also received BCG.

Is There a Need for Intensive Postremission Therapy in AML?

The traditional definition of the term "maintenance therapy" is that of a relatively mild continuation of treatment, having the purpose of "maintaining" complete remission without placing the patient at risk for undue toxic reactions. In large part, this concept evolved from the experience with childhood ALL in which such an approach was quite successful. Inserting the same principle into the management of patients with AML, while resulting in somewhat longer remissions than those in unmaintained groups, has not led to median remission durations > 12–15 months. One of the possible explanations for this observation, as noted previously, is the decreased sensitivity of the leukemic myeloblast to chemotherapy. If high-dose treatment resulting in marrow aplasia is required to induce a complete remission in AML, it seems highly unlikely that the number of residual leukemic cells will be further reduced or even stabilized by the use of relatively nonmyelosuppressive, intermittent, maintenance treatment.

Because of the narrow therapeutic index of cytotoxic agents in the treatment of AML, the drug doses required to kill leukemic myeloblasts during remission induction severely suppress normal hematopoietic progenitors, leading to a period of pancytopenia. It is reasonable to assume that similar myelosuppressive treatment will be necessary after remission is achieved to further reduce the leukemic cell burden. The use of such intensive therapy during remission, even at doses greater than those administered during induction, takes advantage of the steep dose-response curve that has been demonstrated for chemotherapeutic agents such as cytarabine in experimental tumors (29) and in human leukemic cells in tissue culture (30). A linear-logarithmic relationship between dose and reduction of tumor cells has been shown in these systems (29,30).The administration of such high-dose maintenance treatment is also consistent with the proposal that intensification may be necessary to eradicate leukemia at a time (ie, remission) when the leukemic cell burden has been reduced (31).

Two recent clinical trials (27,28) (included in table 2) have utilized the principle of intensive maintenance therapy in groups of young patients. One study (27), from the Sidney Farber Cancer Institute and known as VAPA, included 16 courses of sequential chemotherapy, with cytarabine being given in eight of these courses by continuous administration at a dose (200 mg/m^2/day) higher than that used during remission induction. The second trial (28), conducted at Roswell Park Memorial Institute, offered patients three courses of high-dose consolidation treatment followed by a sequential maintenance program. The median duration of remission and the percent of patients remaining in remission after 12 months in these studies are higher than those noted in previous investigations (table 2).

The VAPA Experience: An Update

A total of 107 consecutive previously untreated patients < 50 years of age with AML were entered in this study between February 1976 and May 1980. The experimental design and the preliminary results in the first 83 patients have been reported previously (27). The median age for the entire group

TABLE 3.—Update of VAPA experience

	No. of patients by age		
	0–50 yrs (median, 14.5)	0–17 yrs (median, 9.5)	18–50 yrs (median, 34.5)
Entered in study	107	61	46
Complete remissions	75 (70%)	45 (74%)	30 (65%)
Withdrawals	8	5	3
Deaths during remission	2	0	2
Relapses	32	18	14
Bone marrow	21	9	12
CNS	9	8	1
Myeloblastoma	2	1	1
Continuous complete remission	33	22	11
Completed therapy	37	25	12
Relapses while not receiving therapy	8	4	4

was 14.5 years, reflecting the pattern of patient referral. An analysis of this experience as of October 1, 1981, is shown in table 3. Seventy-five of the 107 patients (70%) achieved complete remission with an induction program that included 3 days of an anthracycline (doxorubicin) and 7 days of continuous-infusion cytarabine (100 mg/m²/day) with the addition of vincristine and prednisolone. The patients who entered complete remission were then treated with intensive chemotherapy during a 14–15-month maintenance period, with the treatment subdivided into four sequences of drug combinations (table 4). Such intensive treatment was frequently associated with the development of severe thrombocytopenia requiring platelet transfusions and neutropenia accompanied by fevers, necessitating short-term hospitalizations and the use of broad-spectrum antibiotics.

The median duration of followup time for the 75 patients who entered complete remission is now 19 months, and only three patients are still receiving protocol treatment. There have been 32 relapses,

TABLE 4.—VAPA: intensive sequential maintenance schema

Sequence	Drug regimen
I	Doxorubicin, 45 mg/m²/day, Day 1, iv pulse Cytarabine, 200 mg/m²/day, Days 1–5, continuous infusion q3–4 wks × 4
II	Doxorubicin, 30 mg/m²/day, Day 1, iv pulse Azacitidine, 150 mg/m²/day, Days 1–5, continuous infusion q4 wks × 4
III	Vincristine, 1.5 mg/m²/day, Day 1, iv pulse Methylprednisolone, 800 mg/m²/day, Days 1–5, iv pulse 6-Mercaptopurine, 500 mg/m²/day, Days 1–5, iv pulse Methotrexate, 7.5 mg/m²/day, Days 1–5, iv pulse q3 wks × 4
IV	Cytarabine, 200 mg/m²/day, Days 1–5, continuous infusion q3 wks × 4

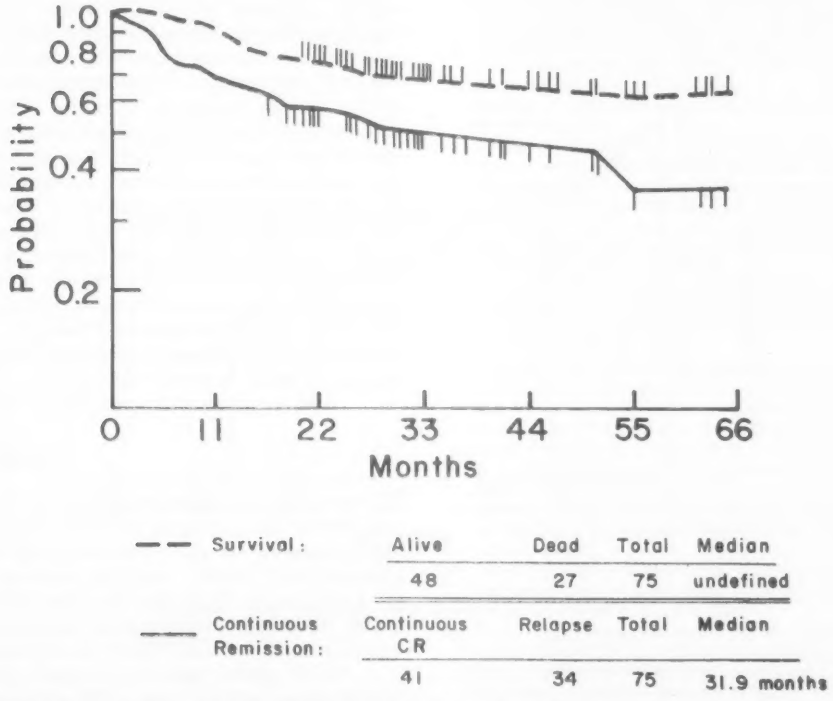


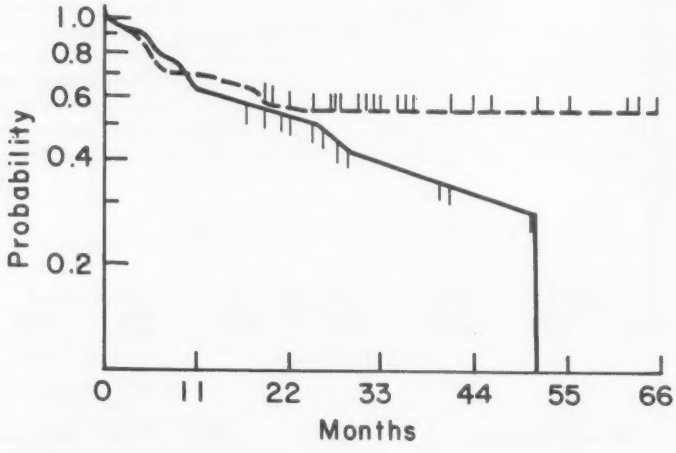
FIGURE 1.—VAPA: Kaplan-Meier plot of probability of survival and probability of remaining in complete remission among all complete responders. Ticks indicate the 40 patients who remain alive and the 33 patients who continue in complete remission. The 8 patients who were "withdrawn" from the study contributed to the curves until the time of their withdrawal.

two deaths during remission, and eight withdrawals during remission. Thirty-three patients remain in continuous complete remission, with the median followup time for this subset being 31 months. Of 37 patients who have completed the sequential maintenance regimen, eight have relapsed after completing therapy while 29 remain in "unmaintained" complete remission. The median duration of followup time after the completion of therapy for this group of 37 patients is 14 months (range, 1+–50+).

Figure 1 presents a Kaplan-Meier plot of the probability of continued complete remission and survival among the patients achieving a complete response. As previously noted, treatment was discontinued at approximately 15 months. The median duration of complete remission is estimated to be 31.9 months, while the median duration of survival is undefined. Utilizing two standard deviation confidence limits (2 SD), the probability for patients achieving a complete response to remain in remission after 12, 24, and 36 months is 68% ± 11%, 55% ± 13%, and 51% ± 18%, respectively.

Figure 2 presents a Kaplan-Meier plot comparing the duration of complete remission in the pediatric and adult subgroups. The median remission duration in the pediatric group is undefined. with 55% ± 13% (2 SD) of patients remaining disease-free at 24 months and no child having relapsed after that time. In the adult group, 50% ± 25% (2 SD) of patients are estimated to remain in complete remission at 26 months. The relatively wide range in 2 SD confidence limits for the adults is a reflection of the small number of these patients in this trial. Additionally, the adult curve is strongly influenced by the development of two late relapses that occurred in a 40-year-old woman and a 21-year-old man, both of whom had completed therapy 36 months previously. The total duration of remission for these two patients was 51 and 55 months, respectively. Such late failures, while unusual, have been observed by others (32).

Reanalysis of the results of the VAPA program, therefore, continue to support the use of intensive postinduction therapy for patients with AML, especially in the pediatric age group. These data furthermore suggest that chemotherapy alone can effectively suppress the leukemic clone for extended periods of time and raise the possibility that a significant number of these patients may be cured of their disease.



Age	Continuous CR	Relapse	Total	Median
0-17 years	22	18	40	undefined
18-50 years	11	16	27	25.6

FIGURE 2.—VAPA: Kaplan-Meier plot of probability of remaining in complete remission, comparing pediatric and adult age groups. Ticks indicate the 22 children and 11 adults who continue in complete remission. The 5 children and 3 adults who were "withdrawn" from the study contributed to the curves until the time of their withdrawal.

Allogeneic Bone Marrow Transplantation: Another Approach to Intensive Maintenance Therapy

Because of the increasing evidence that AML is a clonal disease, possibly representing a malignancy of the pluripotent stem cell (22), and because of the short remission duration associated with nonintensive maintenance chemotherapy, several investigators have directed their efforts at replacing the stem cell pool during first remission through the use of ablative chemotherapy and radiation therapy followed by allogeneic bone marrow transplantation. The initial experience with this form of therapy reported from several transplantation centers (33–35) in patients generally < 35 years of age has been encouraging, with graft-versus-host disease representing the major cause of morbidity and with leukemic relapse being relatively uncommon. Approximately 60% of patients with AML who undergo bone marrow transplantation during their first remission may be expected to be alive 24 months after the graft, and failure thereafter has been uncommon.

In a sense, allogeneic bone marrow transplantation represents the ultimate in "intensive maintenance" therapy. Residual leukemic cells are eliminated by ablative treatment and the stem cell pool is replaced. At the present time, however, this therapeutic option is limited by immunologic considerations. Eligibility for the procedure is restricted to the 30%–40% of patients who have an available histocompatible donor. Even when marrow from such an ideal donor is utilized, graft-versus-host disease will be observed after 50% of transplants (36) and will be a fatal complication in about 33% of the patients (35). A future reduction in the incidence of the chronic form of graft-versus-host disease may result in a limited therapeutic benefit, since this "complication" appears to produce an antileukemic effect (36), presumably through lymphocyte-mediated cytotoxicity toward leukemic cells.

CONCLUSION

The results of aggressive approaches, such as high-dose sequential chemotherapy (eg, the VAPA program) and allogeneic bone marrow transplantation following marrow ablation, indicate that intensive treatment for patients with AML in remission can lead to a prolonged "unmaintained" disease-free state. The comparative merits of these two treatment modalities have been the focus of debate (37).

Patients with AML who receive some form of maintenance chemotherapy remain in complete remission longer than those who do not receive such treatment. The VAPA program and the Roswell Park experience suggest that intensive postinduction treatment may lead to longer durations of remission. Interpretation of these data, however, should take into account the young median ages in both studies. In addition, such issues as the intensity and duration of postinduction chemotherapy remain unsettled. Furthermore, it has yet to be shown in a prospectively randomized fashion that intensive postremission therapy in patients with AML is superior to regimens with less myelosuppressive treatment. Future clinical trials should directly address these issues before any definite conclusions can be made.

At the present time, the emphasis for physicians who treat patients with AML should no longer be limited to a goal of remission induction but should now be expanded to aggressive means of prolonging that remission and achieving a cure.

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Marrow Transplantation for Patients With Acute Nonlymphoblastic Leukemia Who Achieve a First Remission[1,2]

E. Donnall Thomas,* Reginald A. Clift, and C. Dean Buckner[3]

For the Seattle Marrow Transplant Team

Marrow transplantation for patients with acute nonlymphoblastic leukemia (ANL) was first carried out for patients in relapse, most of whom had failed to respond to combination chemotherapy. In 1977 we reported a series of 54 such patients given 60 mg/kg of cyclophosphamide on each of 2 days, followed by 1000 rad of total-body irradiation and marrow grafts from HLA-identical siblings (1). There were many deaths in the first weeks after grafting, due to the complications of advanced illness, graft-versus-host disease, and opportunistic infections. Sixteen patients had a recurrence of leukemia in subsequent months, with the last relapse of leukemia occurring 18 months after transplantation. An actuarial analysis shows that 61% of these patients would have had a relapse of leukemia if there were no other causes of death (fig 1). Six of these patients, who received no antileukemic therapy after grafting, are alive in continuing remission and leading essentially normal lives, now 6–10 years after transplantation. These patients ranged in age from 16 to 36 years at the time of transplantation. Three have had cataract operations and none have chronic graft-versus-host disease. Figure 1 shows the survival curve for these 54 patients and demonstrates that the survivors are far out on a plateau and presumably cured of the disease (2).

In view of the apparent cure of a small fraction of end-stage patients, we turned to transplantation in first remission for patients with ANL (3). The regimen consisted of 60 mg/kg of cyclophosphamide × 2 and 920 rad of total-body irradiation, followed by marrow from an HLA-identical sibling. Ten of the first 19 such patients, reported in 1979, are now living in remission. Three additional patients were treated with this regimen and two are in remission. The median disease-free survival has not been reached but will be ⩾ 44 months from transplantation.

Our next study was designed to evaluate the possibility of improving results by the use of fractionated total-body irradiation (4). Fifty-three patients with ANL in first remission were treated with cyclophosphamide, total-body irradiation, marrow transplantation, and the usual regimen of methotrexate given in the first 100 days after transplantation. Twenty-seven patients were randomized to receive 1000 rad in one exposure, and 26 were given 200 rad on each of 6 days. Eleven of the patients given the single exposure and 15 of the fractionated irradiation recipients are living.

Thus, 75 patients with ANL have been transplanted in first remission, with the survivors followed for 12–58 months. Only six patients have had a recurrence of leukemia. The median time from transplant to relapse was 12 months (range, 6–30). Five of the six relapses occurred within 14 months of transplant. Figure 2 shows a Kaplan-Meier plot (5) of survival and of the probability of being in remission for these 75 patients. One patient died of a pneumothorax while in remission 4.5 years after grafting.

In our studies, as well as in the studies of other marrow transplant teams, emphasis has been placed on transplanting younger patients. Our transplant protocols provide an upper limit of 50 years of age. Figure 3 shows the Kaplan-Meier plot of the probability of survival according to age for the 75 patients with ANL in first remission described above. The fraction of long-term survivors is greater for the younger patients ($P = 0.03$), and the difference in survival appears to be almost entirely related to the increased frequency and severity of graft-versus-host disease in the older patients.

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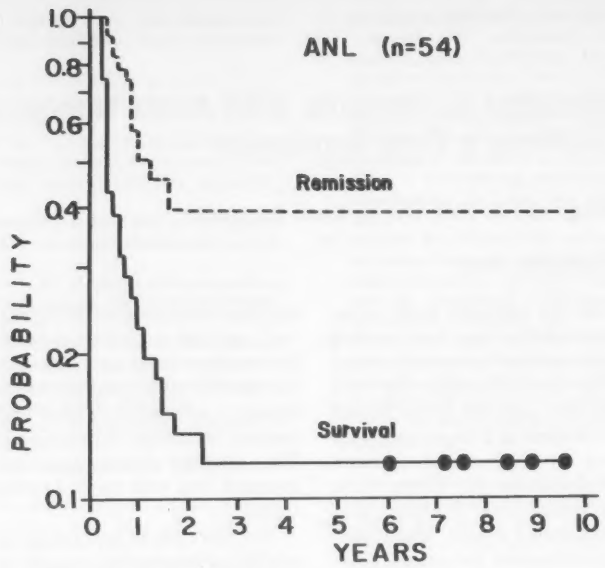

FIGURE 1.—Kaplan-Meier product limit estimates of the probability of survival and of being in remission for 54 patients with ANL who had transplants in relapse. Dots indicate 6 patients living in remission.

DISCUSSION

The actual (not projected) 3-year survival was 59% for the first 22 patients transplanted while in remission described above. The median survival from transplantation will be ≥ 44 months, and the median time from initial diagnosis and treatment will be > 47 months. These figures are superior to any yet reported for patients treated with combination chemotherapy. The actuarial analysis of the subsequent 53 patients and the reports by two other marrow transplant teams (6,7) provide additional evidence of the superiority of the marrow transplant regimens.

A comparison of median survival data is informative but not definitive. The major question is how many patients are actually being cured. The apparent cure of end-stage patients with marrow transplantation, now borne out by 6–10 years of followup, indicates that many of the patients transplanted in first remission are likely to be cured (fig 2). The actuarial analysis indicates that marrow transplantation for patients between 20 and 30 years of age with ANL in first remission will cure slightly more than one half of the patients. For patients < 20 years old, the cure rate will be superior, and for those > 30 years old, the results will be worse (fig 3).

Marrow transplantation is presently the treatment of choice for selected groups of patients only. First of all, the patient must have a suitable marrow donor. Our experience indicates that approximately 40% of the patients will have HLA-identical siblings. Current exploration of transplants using haploidentical family-member donors (8) and unrelated phenotypically HLA-identical donors (9) is encouraging and points the way to a much larger number of potential candidates for marrow transplantation. Second, age is an important factor in considering marrow transplantation for patients with ANL in first remission. Marrow transplantation is currently limited to patients < 50 years old, and the most beneficial results are achieved with patients < 30 years old.

Several marrow transplant centers are conducting studies designed to improve the results of marrow transplantation, including regimens of fractionated irradiation, cyclosporin A, interferon, and monoclonal antibodies. Similarly, attempts are being made to improve the results of combination chemotherapy by the use of new drugs, drug combinations, and immunotherapy. Claims of cure by any

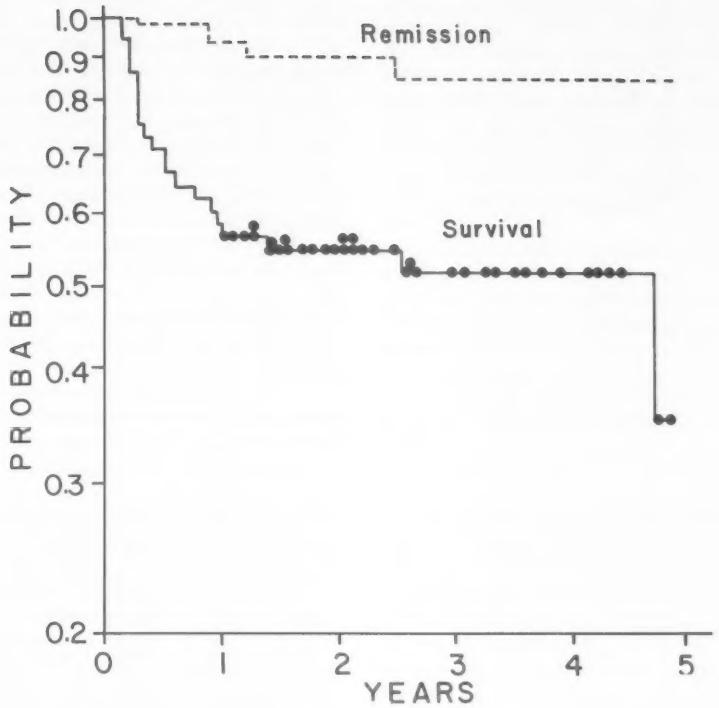

FIGURE 2.—Kaplan-Meier product limit estimates of the probability of survival and of being in remission for 75 patients with ANL who had transplants in 1st remission. Dots indicate 38 patients living in remission.

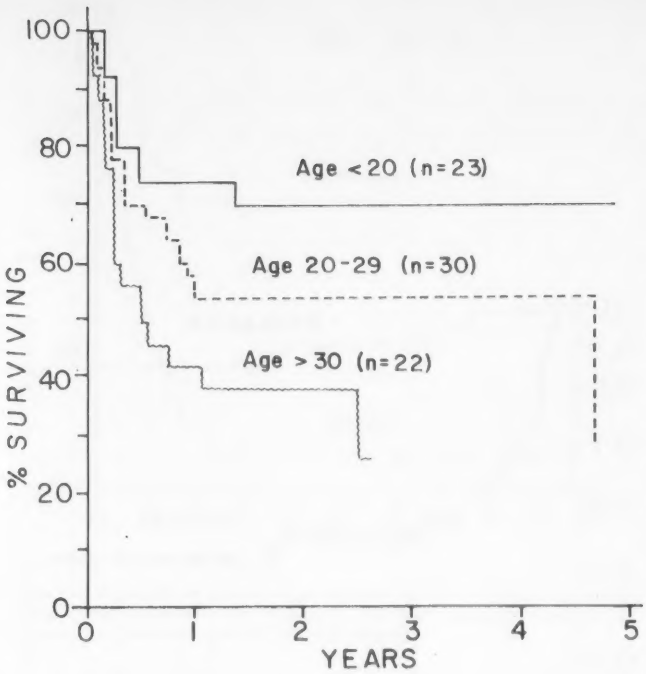

FIGURE 3.—Kaplan-Meier product limit estimates of the probability of survival according to age.

therapeutic regimen must be based on the demonstration of long-term survival after therapy is stopped and on the demonstration of a flat plateau of several years' duration on an actuarial analysis of the probability of being in remission, most evident when these data are plotted on a semilogarithmic scale.

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Therapy for Patients With Acute Myelocytic Leukemia Who Enter Remission: Bone Marrow Transplantation or Chemotherapy?[1,2]

H. D. Preisler[3,*]

Substantial progress has been made in the treatment of young patients with acute myelocytic leukemia. Remission rates for individuals < 30 years of age are very high with, for example, 42 of the last 45 of such patients treated at Roswell Park Memorial Institute entering complete remission (1), a remission rate comparable to that for pediatric acute lymphocytic leukemia. The papers by Drs. Mayer and Thomas and their co-workers (1,2) suggest that substantial progress has also been made with respect to remission duration and survival. The marrow transplantation studies reported by Blume et al (4) and Powles et al (5) provide data that confirm the early results of the Seattle group, while studies of the role of intensive consolidation chemotherapy carried out at Roswell Park provide remission duration curves that are similar to those reported by Mayer and colleagues (1) (fig 1).

A side-by-side comparison of the Mayer and Thomas papers (1,2) suggests both similarities and differences in treatment outcomes. Both studies report the treatment results for 75–80 patients followed for 1–5 years. Comparison of the survival curves and the remission duration curves suggests that significant differences may emerge in the future. The proportion of surviving patients appears to be greater for the chemotherapy study at all points in time. The differences in the slopes of the curves are greatest for the first year, a reflection of the high initial mortality rate for the transplanted patients. In contrast, examination of the relapse curves suggests an advantage for the transplantation approach, since the chemotherapy curve indicates a continuous relapse rate for the duration of the study while the comparable curve for transplanted patients appears to be relatively flat with few relapses. These data reflect the fact that the problems associated with each therapeutic approach are different, with the transplantation studies essentially limited by treatment-related deaths and the chemotherapy approaches limited by the problem of leukemic relapse. Partial solutions of these problems will probably result in a merging of the problems, with more aggressive chemotherapy associated with an increase in disease-free mortality and a decrease in graft-versus-host (GVH) disease perhaps associated with leukemic relapse in transplanted patients becoming more common (see below).

The likelihood of a good therapeutic outcome is age-dependent for both therapeutic approaches. For patients < 18 years old treated with chemotherapy, the relapse-free curve appears to be flat, suggesting a state free of risk of relapse. There are, however, only 22 patients on this curve so that the relapse of even a single patient could significantly alter the shape of the curve. Similarly, the best results for transplantation are for patients who are < 20 years of age, with the survival of patients > 30 years old being projected at only 20%–40% at 2½ years.

A potential advantage for the chemotherapeutic approach is that therapeutic failure currently means leukemic relapse, a treatable event. In contrast, treatment failure for a transplant patient usually means death. Although patients who relapse while receiving chemotherapy probably cannot be cured by currently available chemotherapeutic agents, the Intergroup AML study has found that approximately 50% of these patients will re-enter remission after therapy with a cytarabine/doxorubicin antibiotic regimen and that the current median duration of unmaintained remission is 4¼ months (6). One would expect that patients who relapse after therapy is stopped [as have one half of the relapsers in the Mayer et al study (1)] will be even more likely to re-enter remission and to have a longer duration of remission if they receive consolidation therapy. Therefore, even though the chemotherapy patients appear to be on a continuous relapse curve, the survival curve of the patients who were the subjects of the Mayer report will not necessarily cross that of the transplanted patients because of the likelihood that new approaches to chemotherapy will be available in the future.

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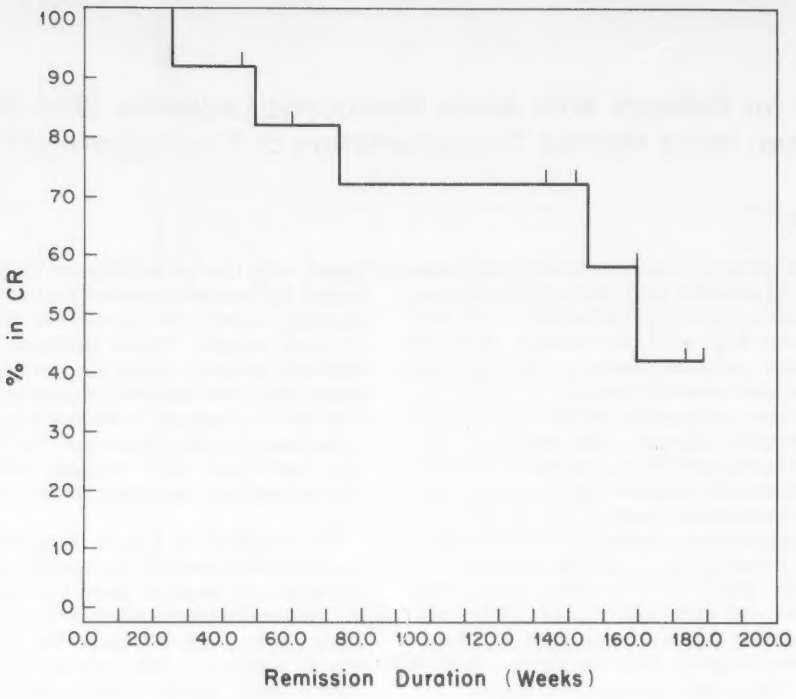

FIGURE 1.—Remission duration curve for patients < 30 yrs of age treated with intensive consolidation chemotherapy. Patients were induced into remission with cytarabine-doxorubicin. Consolidation therapy consisted of: *course 1*—100 mg/m² of cytarabine twice a day × 6 days and 30 mg/m²/day of doxorubicin × 2; *course 2*—100 mg/m² of cytarabine twice a day × 7 days and 500 mg/m² of cyclophosphamide on Day 1; *course 3*—100 mg/m²/day of cytarabine by continuous infusion × 7 days and 30 mg/m²/day of doxorubicin × 3. Once consolidation was completed, patients received daily 6-thioguanine (40–80 mg/day) and weekly methyl-GAG (300 mg/m²). Every 3 mos they received consolidation chemotherapy consisting of an alternation of courses 2 and 3 for 3 yrs.

SPECIFIC COMMENTS ON BONE MARROW TRANSPLANTATION IN FIRST REMISSION

The use of marrow allografting after ablative therapy has several theoretic advantages over the chemotherapeutic approach. In principle, if one could deliver truly ablative therapy without having to leave enough normal hematopoietic stem cells to repopulate the patient's bone marrow, then one could attempt to kill the "last" leukemic cell. As will be discussed below, it is difficult to imagine such definitive therapy being accomplished by the currently available chemotherapeutic agents. Replacement of a patient's hematopoietic stem cells would be particularly important if, as has been speculated, there are no normal residual progenitor cells in leukemic patients (7). Unfortunately, as described below, it does not appear likely that even the currently employed ablative transplant preparative regimens are capable of destroying the "last" leukemic cell. The use of total-body irradiation as part of the transplant preparative regimens has a potential advantage over chemotherapy in that radiation therapy reaches all sanctuary sites independent of various "barriers" that affect drug distribution. This may be particularly important in preventing late relapses, since these appear to be occurring in unusual sites such as the skin, central nervous system, etc—problems reminiscent of pediatric acute lymphocytic leukemia. Since it appears likely that identical twin transplantation is inferior to allogeneic grafting in terms of antileukemic efficacy, despite the

same preparative regimens being used, the long-term disease-free survival associated with allografting must be the result of some additional phenomena.

A claimed advantage of transplantation is that the therapy is administered once and not in repeated courses as is consolidation chemotherapy. This claim must be viewed, however, with some degree of skepticism, since it is likely that GVH disease plays a role in maintaining the transplanted patients in a state in which leukemia is at least not clinically apparent (8,9). Hence, it appears that one of the antileukemia components of transplantation involves a process that persists for a long period of time and that in and of itself may produce late and unexpected toxic effects. Since only a small number of patients have actually been followed for more than several years, there are essentially no data regarding long-term complications, which may include damage to vital organs, second malignancies, and premature aging. The long-term and continuous nature of GVH disease also suggests that patients in long-term remission may not be disease-free but rather that leukemic cells may be present at a subclinical state, with the inherent possibility of late relapse. While the initial experiences with allografting in complete remission are encouraging, it is not as yet known if the relapse-free survival curves are indeed flat after 1–2 years.

Advances in transplantation therapy will depend on overcoming the current age limitations and tissue typing requirements, which limit the availability of this approach. Posttransplantation complications due to GVH disease and infection must be reduced but it should be realized, however, that a reduction in GVH disease may be associated with an increase in the rate of leukemic relapse. The immediate and perhaps long-term complications of the transplantation preparative procedure would be reduced if less intensive preparative regimens could be employed, a possibility raised by advances in assays predictive of drug responsiveness in leukemia (10,11).

CHEMOTHERAPY

Chemotherapy, as a means to treat patients in remission, has an advantage over transplantation in that it can be altered in time to suit the needs of the patient. One might also expect that chemotherapy would be less likely to produce long-term complications than does total-body irradiation and/or the administration of pharmacologic doses of alkylating agents. Whether this expectation is valid is unknown.

A major problem in comparing chemotherapy with allogeneic transplantation as "curative modalities" lies in the fact that there is no standard postremission chemotherapeutic regimen that can be compared to the relatively standardized transplantation regimens. The various studies of the efficacy of transplantation are relatively consistent in reporting a 30%–50% death rate during the first 1–2 years, with an apparent plateau after 2 years and little problem with leukemic relapse (4,5,12). In contrast, median remission duration and the percent long-term survivors among chemotherapy patients vary widely, ranging from short remissions with a few long-term survivors (13–15) to the data presented here. Which, if any, studies should be used to represent the overall efficacy of chemotherapy is unknown. Are the excellent results reported by Mayer et al (1) statistical anomalies or do they represent an advance in therapy? The Boston and Buffalo regimens differ from most other regimens in the intensity of consolidation therapy, and one would therefore like to believe that their superior results are truly reflective of the therapeutic approach and not merely chance observations. It is clear, however, that controlled trials comparing these intensive chemotherapeutic approaches with the more conventional chemotherapeutic programs are necessary.

A variety of "maintenance" and "consolidation" regimens are described in the literature. Some employ intensive therapy, while others are more "gentle." While the median durations of remission are reported, insufficient attention is paid to the percent of disease-free survivors at each year. Additionally, a comparison of the regimens per se is essentially impossible because of the large variety of chemotherapeutic agents used as well as the variety of schedules and routes of administration employed. One group of investigators may report the results of an "intensive consolidation" regimen, which in fact is less intensive than a "maintenance" regimen used by another group of investigators. To obviate this problem, consolidation or maintenance should be reported in terms of both the nadir and the duration of pancytopenia produced, the frequency at which the pancytopenia is produced, and the duration of therapy. Since different courses of therapy are administered and these produce different degrees of toxicity, perhaps the total time spent during each year at different levels of granulocytopenia and thrombocytopenia should be reported.

This approach to reporting treatment regimens would permit a comparison of different regimens in terms of intensity of therapy. The production of hematopoietic toxicity, however, is not synonymous with the production of an antileukemic effect, since there are many agents that are potent suppressors of normal hematopoiesis but have little or no anti-

leukemic efficacy. Therefore, some effort should be made to rate treatment regimens in terms of their potential antileukemic efficacy. Perhaps chemotherapeutic agents employed in the regimens should be rated in terms of their antileukemic efficacy when administered as single agents. Agents that produce a complete remission rate of 20% could be class A drugs, those that produce 10%–20% complete remissions could be class B drugs, and those that produce < 10% complete remissions could be class C drugs. If the total doses of such rated agents administered (perhaps normalized to their optimum dose levels when used as single agents) were reported together with the frequency of administration, there would be some means for comparing the potential antileukemic efficacy of the different consolidation or maintenance regimens. Clearly, this approach is only a first approximation because of the complexities of drug interactions, dose-level effects, etc.

Recent studies at Roswell Park suggest that leukemic relapse is a complex event in that some patients relapse with drug-resistant disease while others relapse with leukemic cells that are sensitive to the drug with which they are being treated (16). While the former situation was expected, the latter was not. Taken together with the observation that plasma doxorubicin levels may become progressively lower in patients who are repeatedly treated with this agent (17), it is possible that on some occasions leukemic relapse occurs because of inadequate delivery of drug to the target cells in vivo. Leukemic relapse with drug-sensitive cells can also occur in the setting where patients cannot, for hematologic reasons, tolerate frequent or intensive courses of consolidation or maintenance therapy. Some of the latter patients should probably be considered for marrow transplantation once their inability to tolerate therapy is documented, while pharmacologic monitoring, with dosage escalation to insure the delivery of effective doses of chemotherapeutic agents during consolidation therapy, might prevent relapse of the former patients.

CONCLUSIONS

Both allogeneic marrow transplantation and intensive chemotherapy would be more effective if it were possible to tailor the treatment regimens to the characteristics of the individual patients. The data represented in figure 2A illustrate a typical dose-response curve for the sensitivity of leukemic cells to cytarabine, with exposure to increasing concentration of drug producing increasing cell death. Figure 2B demonstrates that the curve in figure 2A is really a mean population curve and does not represent the drug sensitivity curves of the individual patients. Substantial differences in the drug sensitivity of the leukemic cells of different patients suggest that the intensity and frequency of ablative therapy and/or consolidation therapy as well as the selection of the agents to be utilized should be determined by the sensitivity patterns of the leukemic cells, so that specific patients will not be overtreated or undertreated. Avoidance of the former would reduce the morbidity and lethality of therapy, while avoidance of the latter would reduce the incidence of leukemic relapse.

Little is known about the causes of late leukemic relapse. As noted above, it is difficult to imagine that any of the currently available chemotherapeutic agents are sufficiently selective in their effects to be able to kill the "last" leukemic cell while permitting a sufficient number of normal hematopoietic stem cells to survive. If this view is correct, then how can one account for long-term remissions and perhaps cures? Are there immunologic mechanisms capable of destroying or restraining the proliferation of leukemic cells? Is GVH disease a model of this phenomenon or are the possible effects of the graft-versus-leukemia reaction merely a manifestation of a GVH (in this case versus host hematopoietic tissue) reaction? Do chemotherapeutic agents restore the responsiveness of leukemic cells to mechanisms that normally regulate cell proliferation and differentiation? If these regulatory phenomena exist and play a role in the maintenance of long-term responses, are they manifestations of the effects of therapy on the leukemic cells or on the host? If there are normal host restraints, why does leukemia ever become clinically apparent? Do late recurrences actually represent the appearance of a second leukemia?

A comparison of allogeneic marrow transplantation during remission with intensive consolidation chemotherapy is also complicated by the fact that a chemotherapeutic regimen which produces a mortality rate (50% in the first year) comparable to that of the transplantation regimen has not as yet been administered nor is it likely to be. It appears to be more psychologically acceptable for this mortality rate to be produced as a result of transplantation than chemotherapy. While this acceptance is based at least in part on the curative potential of transplantation (18), it should be remembered that most modern chemotherapeutic regimens of even moderate intensity produce 10%–20% long-term survivors, suggesting that increases in the intensity of such therapy are indeed warranted. Unless or until chemotherapeutic regimens of comparable toxicity

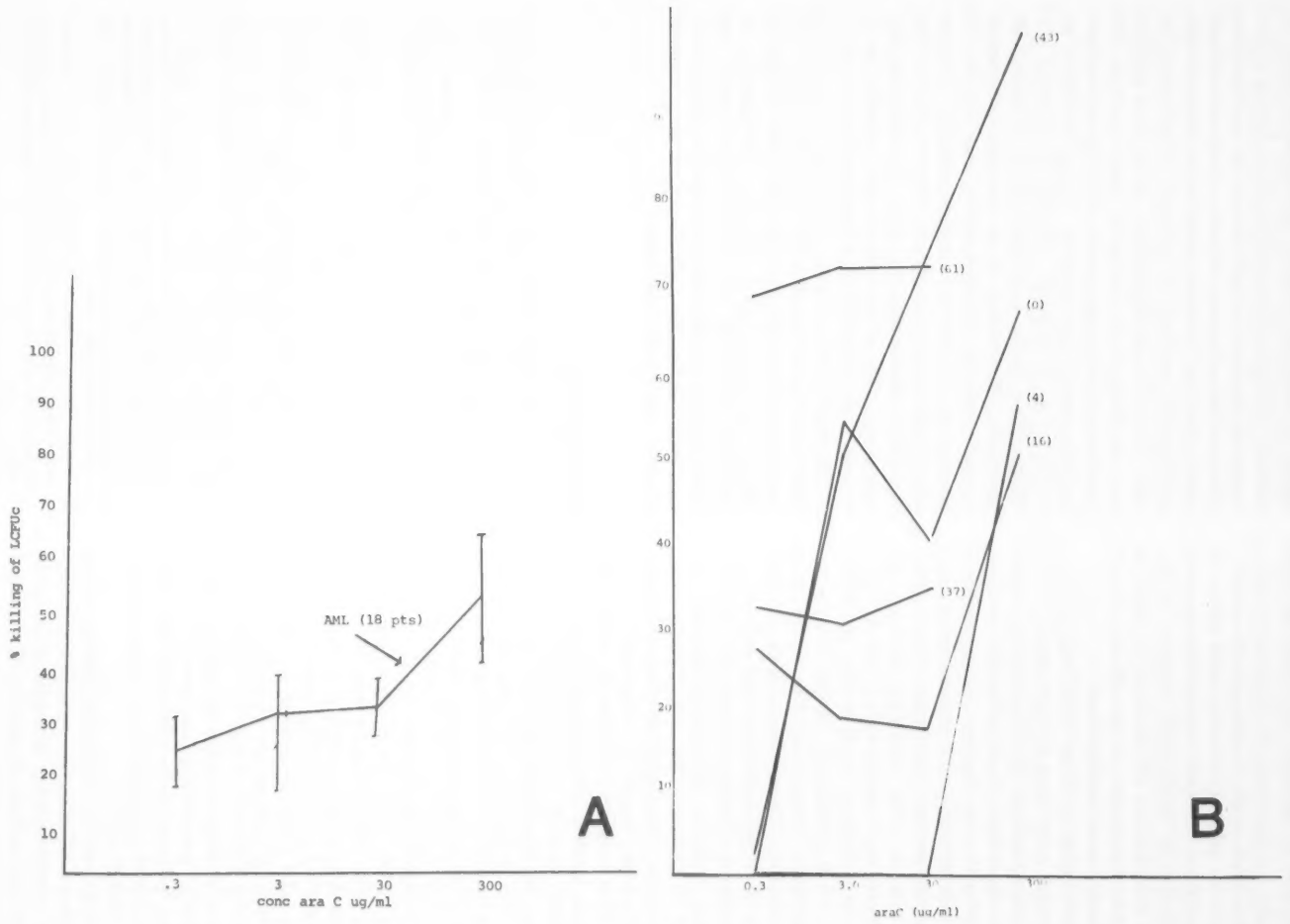

FIGURE 2.—Sensitivity of clonogenic leukemic cells (LCFUc) to cytarabine (ara C) in vitro. (A) Relationship between concentration of ara C and killing of LCFUc for 18 leukemic patients. Methods described in ref 6. (B) Relationship between concentration of ara C and killing of LCFUc from perspective of ara C sensitivity of LCFUc of individual patients. These data form part of pooled data represented in (A) above. Note that LCFUc of some patients are very sensitive to lowest ara C concentrations, while others are only killed by exposure to high ara C levels.

are administered to patients, a true comparison of the ability of the transplantation and chemotherapeutic approaches to produce long-term survival cannot be made. Furthermore, to the present time, no attempt has been made to compare the quality of life for patients who are treated with either of these two modalities. As each therapeutic approval becomes available for increasing numbers of patients, these comparisons will have to be made as well and put into perspective as the efficacy of each approach becomes more well-defined.

There is developing evidence that long-term remission can result even from a single course of remission induction therapy (19,20) and that second remissions are more likely to be induced in patients who relapse after therapy has been stopped than in patients who relapse while receiving chemotherapy. As already noted, second remissions, even in individuals who relapse while receiving therapy and who do not receive any consolidation or maintenance therapy, can be prolonged, with median durations in the range of 4–5 months (6). In this setting, intensive consolidation therapy might in fact produce long-term remissions. These observations suggest the possibility of an approach that takes advantage of the best features of chemotherapy and marrow transplantation. Patients who enter complete remission could be treated with only three or four courses of intensive consolidation chemotherapy. Patients who relapse would be reinduced into remission and then grafted if a suitable donor was available. While marrow allografting of patients in second remission appears to be associated with a higher relapse rate than when patients are grafted during their first remission, this may not be the case for patients who are in second remission after having relapsed while not receiving therapy, since these patients are less likely to have drug-resistant disease than patients who relapse while receiving chemotherapy. Older patients or those without a suitable donor who relapse could then be treated with more intensive or more prolonged chemotherapy than when they were initially treated. In this way a clinical trial could be carried out aimed at recognizing those individuals for whom the morbidity and mortality inherent in marrow transplantation or intensive prolonged chemotherapy is worthwhile. Hopefully, pretherapy assessment of the patient and his leukemic cells will reach the point that will permit such a decision to be made without conducting a clinical trial.

At the present time it is not known if allografting during remission and intensive consolidation chemotherapy are complementary therapies or are most suitable for the same subset of patients. Specialized facilities and physicians with specialized experience are necessary to effectively administer either of these therapeutic modalities, and when possible, patients should be referred to these specialized facilities for therapy. Allogeneic marrow grafting during clinical complete remission would seem to be the treatment of choice for patients in whom a cytogenetic abnormality that had been detected in the leukemic cells at diagnosis persists or increases in frequency despite intensive chemotherapy, since these patients are not likely to be cured by chemotherapy. The persistence and progression of cytogenetic abnormalities despite an apparent clinical remission appears to be particularly common in patients with Ph^1-positive acute leukemia (H. D. Preisler, M. Marinello, M. Brecher, et al, manuscript in preparation). Marrow transplantation may also be the treatment of choice for younger patients (< 20 years of age) who do not have ready access to centers that can safely administer intensive consolidation chemotherapy. These patients should probably be referred to an established transplantation center.

The treatment outcome for young patients who receive intensive consolidation chemotherapy or who receive an allogeneic marrow transplant in first remission is encouraging. Neither therapeutic approach is presently the treatment of choice, and both short-term and long-term studies must be carried out to define the role of each in the treatment of acute myelocytic leukemia.

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AMSA—A Promising New Agent in Refractory Acute Leukemia[1,2,3]

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SUMMARY

Forty-five patients with various forms of acute leukemia refractory to usual methods of treatment were treated with AMSA at doses of 100–200 mg/m² iv daily for 5–7 days, for total doses of 500–1000 mg/m²/course. Among 41 evaluable patients, six (15%) achieved complete remissions, including two of 19 patients with typical acute nonlymphocytic leukemia, none of four with atypical acute nonlymphocytic leukemia, two of nine with chronic granulocytic leukemia in blast crisis, two of eight with acute lymphoblastic leukemia, and none of one with acute undifferentiated leukemia. Durations of complete remissions were 6, 7, 8, 12, 13, and 14 weeks. Four of the patients who died with infection during marrow hypoplasia 2–5 weeks after receiving AMSA had no evidence of leukemia on premortem bone marrow aspirates or postmortem examination. The primary toxic effects of AMSA were severe myelosuppression, stomatitis, and alopecia. One incident of life-threatening liver failure occurred. AMSA appears to be a promising agent for use in heavily pretreated patients with acute leukemia and chronic granulocytic leukemia in blast crisis.

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AMSA is an aminoacridine dicationic analog of the 4′-(9-acridinylamino) alkanesulfon anilides with a wide spectrum of antitumor activity in animal models (1). As one of several similar compounds, AMSA was chosen for study in human subjects primarily because of its superior activity in L1210 leukemia. (1).

The primary mechanism of the antitumor effect of AMSA is probably intercalation with DNA, a mechanism similar to that of anthracyclines (2). The drug is detoxified in the liver by conjugation with glutathione and excretion into the bile (3). Pharmacokinetic studies in humans with normal renal and hepatic function have shown that plasma disappearance of AMSA is biphasic, with a terminal-phase half-life of 7.4 hours (4). The half-life was mildly prolonged in a patient with severe renal failure (10.8 hours) and markedly prolonged in patients with severe hepatic dysfunction (17.5 hours).

Phase I studies have shown myelosuppression, stomatitis, and alopecia to be the most frequent toxic effects (5–8). Nausea, vomiting, and diarrhea have usually been mild. Rare cases of generalized convulsions and cardiac arrhythmias (some with sudden death) have been reported (9).

Responses in early clinical studies were primarily limited to patients with breast cancer, melanoma, and acute nonlymphocytic leukemia (ANLL) (8,10,11). The responses in ANLL included some complete remissions (CRs) (8), a finding that has fostered increasing interest in using AMSA for pa-

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tients with refractory acute leukemia. Preliminary studies have neither fully explored the range of AMSA activity in different types of leukemia nor clarified the optimal dose schedule. However, a report from the M. D. Anderson Hospital and Tumor Institute (Houston, TX) suggests that a total dose of approximately 525–630 mg/m²/course may be optimal (12).

The following report reviews the experience of the Northern California Oncology Group using three different doses of AMSA in a group of 45 heavily pretreated patients with several forms of acute leukemia.

MATERIALS AND METHODS

From June 1979 to December 1980, 45 patients with various forms of refractory acute leukemia were treated with AMSA. One additional patient was registered on the protocol but died before receiving his first dose. Four patients (three with ANLL and one with chronic granulocytic leukemia in blast crisis [CGL-BC]) were considered inevaluable for response, three because of major protocol violations and one because of incomplete data. The general characteristics of the treated group and the subtypes of leukemia are shown in table 1.

Patients were eligible for this phase II study if they had ANLL, smoldering leukemia, acute undifferentiated leukemia (AUL), acute lymphoblastic leukemia (ALL), or CGL-BC. All patients with ANLL had received at least two courses of cytarabine and anthracyclines. Patients with ALL and AUL had received several courses of vincristine and prednisone as well as anthracyclines. All patients with CGL-BC had received at least one prior course of chemotherapy consisting of hydroxyurea, vincristine, prednisone, 6-mercaptopurine, and anthracyclines in various combinations. All patients signed informed consent papers.

TABLE 1.—General patient characteristics

Characteristic	No. of patients
Total	45
Age (yrs)	
Mean: 36.1	
Range: 9–69	
Sex	
Male	27
Female	18
Histologic type	
Typical ANLL	22
Acute granulocytic (FAB M1 and M2)	17
Acute promyelocytic (FAB M3)	1
Acute myelomonocytic (FAB M4)	4
Atypical ANLL	4
ANLL arising from preleukemia	2
Smoldering ANLL	2
CGL-BC	10
ALL	8
AUL	1
Karnofsky performance status (%)	
10–50	12
60–100	32
Not given	1

Physical examinations and blood cell counts were performed daily during induction therapy and at least weekly following induction until maximum response was determined. Bone marrow aspirations were obtained prior to initiation of treatment and every 1–2 weeks after induction therapy was completed until the maximum response was determined. For patients in CR, blood cell counts were obtained every 3 weeks and bone marrow aspirations every 6–9 weeks.

CR was defined as < 5% blast infiltration of the marrow, a peripheral granulocyte count > 2000/mm³, a platelet count > 100,000/mm³, and no symptoms related to leukemia. Partial remission (PR) was defined by any combination of (*a*) a 5%–25% blast infiltration of the marrow, (*b*) a granulocyte count of 1000–2000/mm³, (*c*) a platelet count of 50,000–100,000/mm³, and (*d*) 1%–5% circulating blasts.

AMSA was given in three different dose schedules: 100 mg/m² daily × 5 days, 200 mg/m² daily × 5 days, and 120 mg/m² daily × 7 days, with dose reductions for pre-existing liver dysfunction. The drug was supplied by the National Cancer Institute, Bethesda, MD, in vials containing 75 mg of AMSA. The contents of each vial were mixed with 13.5 ml of a lactic acid diluent provided with the drug. The mixed solution of AMSA and lactic acid was diluted in a minimum of 250 ml of 5% dextrose in water and infused through an iv line over 30–60 minutes (approximately 2–3 mg/m²/minute). Most patients were hospitalized during the induction therapy and throughout the subsequent period of hypoplasia. Patients entering remission were given AMSA maintenance therapy at a dose of 120 mg/m² every 3 weeks on an outpatient basis.

RESULTS

Response

A prompt antileukemic effect was observed in nearly every patient. In marrow aspirates obtained 1 week after completion of therapy, hypoplasia was universally observed, with marrow cellularities of ≤ 20%. The overall response rate for 41 evaluable patients was 29% (six CRs and six PRs). Responses were distributed among histologic types as shown

TABLE 2.—Best overall response for evaluable patients by type of leukemia

Response	No. of patients				
	ANLL	ALL	CGL-BC	AUL	Total
CR	2	2	2	0	5
PR	5	1	0	0	7
No response	11	2	2	1	16
Early death	5	3	5	0	13
Total	23	8	9	1	41

in table 2. CRs were achieved in two of 19 patients (11%) with typical ANLL, none of four with atypical ANLL (two cases of smoldering leukemia and two cases of ANLL arising from a clear-cut myelodysplastic syndrome), two of eight (25%) with ALL, and two of nine (22%) with CGL-BC. CRs were observed in none of five patients who received cycles of < 600 mg/m² total dose of AMSA, in four of 27 (15%) given 600–850 mg/m², and two of nine (22%) treated with > 850 mg/m². Thirteen patients died during marrow hypoplasia within 4 weeks of treatment (early deaths), and four had no evidence of leukemia on premortem marrow aspiration or autopsy examination.

The two CRs in patients with typical ANLL were achieved with total doses of 800 and 840 mg/m² and lasted through 13 and 14 weeks of maintenance therapy. The two CRs in patients with ALL were accomplished with doses of 840 and 1000 mg/m² and lasted 6 and 8 weeks. The patient with an 8-week CR had T-cell ALL which relapsed after maintenance therapy was withheld because of unusual toxic effects (*see* below). Two patients with CGL-BC achieved CRs after receiving 840 and 1000 mg/m², with return to stable phase of CGL and normalization of peripheral counts. One patient refused all maintenance therapy and relapsed in 12 weeks; the other relapsed in 7 weeks. All CRs were achieved after one cycle of therapy. Five patients received two cycles each without response. Of note were two patients with prior CNS involvement who developed CNS relapses within 1 month of AMSA therapy. Median survival was 9 weeks for the entire group, 28 weeks for complete responders, 21 weeks for partial responders, and 10 weeks for nonresponders, excluding those dying during hypoplasia in the first 4 weeks. This difference in survival times by response levels was statistically significant ($P < 0.001$). There was no significant difference ($P = 0.13$) in survival by histology (ANLL, ALL, and CGL-BC).

Toxicity

Side effects were substantial. All patients developed severe myelosuppression, with marrow recovery occurring in 3–5 weeks. Every patient required transfusions of rbcs and/or platelets. One patient died 4 weeks after his second course of AMSA, with sepsis and persistent marrow hypoplasia.

Severe stomatitis was noted in 18 patients, including one case of life-threatening severity. The frequency of severe stomatitis was dose-related. Severe stomatitis occurred in no courses of < 600 mg/m², 12 of 36 courses of 600–850 mg/m², and six of ten courses of > 850 mg/m². The stomatitis was characterized by painful ulceration and patchy necrosis of the oropharyngeal mucosa with a predilection in some cases for the lateral margins of the tongue. Three instances of moderately severe diarrhea were observed. Nausea and vomiting were usually mild, with only four cases of severe emesis.

Hyperbilirubinemia (serum bilirubin > 1.0 mg/dl) was noted in 23 cycles of therapy, with a range of serum bilirubin levels of 1.1–25.6 mg/dl (median, 2.5). Four patients had bilirubin elevations > 10 mg/dl. Levels of serum alkaline phosphatase of greater than twice normal (100 IU/liter) were noted in 16 cases, but in no patient exceeded 500 IU/liter.

In the patient with T-cell ALL, the SGOT increased rapidly to 675 U/liter 4 weeks after a course of 1000 mg/m² of AMSA. One week later, when the SGOT had decreased to 480 U/liter, the patient received two daily doses of 100 mg/m² of AMSA as maintenance therapy. The third day he presented with jaundice and a serum bilirubin of 6.6 mg/dl. Surface antigen for hepatitis B virus was not detectable. Jaundice, hyperbilirubinuria, and marked transaminasemia persisted for 2 weeks, when percutaneous liver biopsy revealed severe centrilobular necrosis and fatty degeneration. AMSA was withheld but the patient developed progressive hepatic dysfunction, with a maximum serum bilirubin of 21.7 mg/dl, a maximum SGOT of 885 U/liter, and markedly prolonged prothrombin time. Three months after receiving his first course of AMSA, the patient relapsed and died.

Other toxic effects included marked alopecia (all patients), mild to moderate phlebitis (two), and possible mild hearing loss (one). One case of congestive

heart failure was observed, but no serious arrhythmias were noted. No significant neurologic or renal toxic effects were seen.

DISCUSSION

This study demonstrates that AMSA can produce CRs in heavily pretreated patients with ANLL, ALL, or CGL-BC. The response rate in patients with ANLL is comparable to that obtained with 5-azacitidine, an agent commonly used in refractory ANLL but having very little activity in ALL (13). Moreover, AMSA showed activity in two particularly refractory types of leukemia, ie, T-cell ALL and CGL-BC.

The response rate of ANLL in this study was less than the 30% CR rate reported in the M. D. Anderson series (12). However, eight of their 11 CRs were in patients with only one prior treatment regimen. All but five of our patients had received two or more courses of chemotherapy before entry in this study. Moreover, unlike the M. D. Anderson series, we observed CRs in ALL.

The optimal dose and schedule of AMSA are uncertain. One group of investigators had previously reported CRs with total doses of approximately 500 mg/m^2 (8); however, we observed no responses at this level. We therefore increased the dose using two different schedules (200 mg/m^2/day $\times$ 5 days and 120 mg/m^2/day $\times$ 7 days). The toxic effects of these relatively high-dose regimens, particularly stomatitis, were substantial and at the highest dose tested (1000 mg/m^2) were unacceptable. Thus our experience suggests that the optimal dose may be approximately 800 mg/m^2 given over 5–7 days. Even at this "intermediate" dose, myelosuppression is severe and often prolonged (3–5 weeks), with most patients requiring intensive support for many days after treatment.

Severe liver dysfunction, as seen in the patient with T-cell ALL, has not been previously reported with AMSA, perhaps because other studies have used lower doses of the drug. Preclinical studies in dogs, however, have shown significant hepatotoxicity (14). The evidence in this case is circumstantial but suggests at least a contributive role for AMSA in the hepatic failure of this patient. It is also pertinent that many of our other patients developed transient elevations of serum bilirubin and alkaline phosphatase.

In our study, iv AMSA did not confer protection against CNS relapse. This observation confirms the findings of pharmacokinetic studies that only very low spinal fluid concentrations of the drug can be obtained from iv administration (4).

The role of AMSA in maintenance therapy is unclear, although the short duration of remission in this study suggests little benefit from the drug given once every 3 weeks. Nevertheless, AMSA is now being tested at several centers in combination with other agents such as 5-azacitidine and cytarabine in an effort to produce higher response rates in refractory leukemia. Preliminary results are promising (15,16).

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Clinical Evaluation of Succinylated *Acinetobacter* Glutaminase-Asparaginase in Adult Leukemia[1,2]

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SUMMARY

We treated 13 adult patients with acute leukemia or chronic myelocytic leukemia (CML) in blast phase using succinylated *Acinetobacter* glutaminase-asparaginase (SAGA) administered on a daily dose schedule. SAGA reduced the peripheral blast count in two patients with acute lymphoblastic leukemia and two with blastic CML; however, no patient achieved either complete or partial remission. Marked central nervous system toxic effects (encephalopathy and coma) were observed, limiting treatment in patients whose disease appeared responsive; this effect finally prompted early discontinuance of the trial. Other toxic effects observed included nausea, hyperglycemia, and respiratory alkalosis. Hypersensitivity reactions to the enzyme were not seen. Pharmacologic analyses showed that prolonged blood glutamine depletion was achieved only by daily enzyme administration; however, we noted the importance of performing amino acid analysis on blood which was deproteinized immediately following phlebotomy. Our results demonstrate excessive central nervous system toxicity when glutaminase-asparaginase is administered on a daily schedule. Because of this effect, we propose that future trials of similar enzymes be limited to short courses of enzyme therapy, possibly with the addition of antimetabolites or amino acid analogs, which could enhance the antitumor effect without increasing toxicity.

[Cancer Treat Rep 66:1479–1485, 1982]

Enzymatic depletion of "nonessential" amino acids exploits an important biologic difference between normal and neoplastic cells, namely, the inability of some tumor cells to synthesize certain amino acids when deprived of exogenous supplies. The first such enzyme introduced clinically, *Escherichia coli* asparaginase, has substantial activity as a single agent in acute lymphoblastic leukemia (ALL) (1). Furthermore, Rausen et al (2) have shown that the addition of asparaginase to standard chemotherapy markedly increases the complete remission rate achieved in relapsed patients, thus confirming the earlier preclinical observations of Burchenal (3). Moreover, Capizzi (4) demonstrated that, depending upon the timing of enzyme administration, asparaginase can either enhance or negate the therapeutic effects of methotrexate. Several trials have since reported the usefulness of sequential methotrexate-asparaginase chemotherapy for patients with refractory leukemia (5,6).

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The clinical use of asparaginase is limited by its toxicity and by its lack of significant antitumor activity in other disorders (2,7). Also, the development of hypersensitivity and the rapid induction of acquired resistance to the enzyme represent additional problems for patients whose disease may initially be susceptible (8). The glutaminase-asparaginase enzyme isolated from *Acinetobacter* has a much broader spectrum of antitumor activity in vitro than *E. coli* asparaginase (9,10); furthermore, tumor cell lines initially sensitive to this enzyme do not acquire resistance after retreatment (11). While the native enzyme is too rapidly cleared from plasma to be of clinical utility, Holcenberg et al (12) showed that succinylation of the enzyme molecule markedly prolongs the plasma half-life without affecting biologic potency (12).

Our initial clinical evaluation of succinylated *Acinetobacter* glutaminase-asparaginase (SAGA) showed that bolus iv doses only transiently lowered blood glutamine to nondetectable levels (< 5 μM), with rapid recovery thereafter owing to influx of glutamine from extravascular sources. Several doses administered daily were required to achieve prolonged blood glutamine depletion (13). The dose-limiting toxic effect of this schedule was central nervous system (CNS) depression. This effect, however, was dose-related and completely reversible. Moreover, it appeared that potentially severe CNS reactions could be avoided by withholding therapy if asterixis became clinically evident. A daily dose of 320 IU/m^2 was tolerated by most patients and produced some evidence of antileukemic effect. This report summarizes our subsequent experience with this enzyme in the treatment of adult patients with leukemia.

PATIENTS AND METHODS

Patients

Adults with acute leukemia or chronic myelocytic leukemia (CML) in blast crisis were eligible for entry into this study after they failed standard treatment for their disease. All patients had recovered from the toxic effects of previous chemotherapy and had adequate hepatic and renal functions (serum bilirubin and creatinine $\leq$ 1.5 mg/100 ml). Pretreatment evaluation included a bone marrow aspirate, cbc and platelet count, screening biochemical profile, urinalysis, electrocardiogram, and chest roentgenogram. Patients were monitored with the following laboratory tests: cbc and platelet count, daily; serum electrolytes, BUN, and glucose, every other day; and screening biochemical profile and serum amylase, weekly. Serial coagulation tests (prothrombin time, partial thromboplastin time, thrombin time, reptilase time, fibrinogen, fibrin degradation products, and factor VIII coagulant activity) were performed every 3 days. On selected patients, serial determinations of plasma glutaminase activity and whole-blood concentrations of glutamine, glutamate, and ammonia were made according to methods described previously (13). Blood samples used for the latter determinations were deproteinized immediately after phlebotomy by the addition of cold perchloric acid to whole blood (final perchloric acid concentration, 10%). Signed informed consent was obtained in advance and the study was approved by the Clinical Investigations Committee of the Memorial Sloan-Kettering Cancer Center.

Treatment Protocol

Each patient had a skin test by the intradermal injection of 0.1 IU of enzyme/kg of body weight. If neither erythema nor urticaria appeared after 1 hour of observation, the prescribed dose of SAGA was infused iv over 15–20 minutes. All treatment was administered on an inpatient basis.

Therapy was initiated at a daily dose of 300 IU/m^2; treatment was withheld if mental confusion or asterixis developed and was resumed when these signs disappeared. In the absence of CNS toxicity, the dose was escalated by 100 IU/m^2/day every 2–3 days to tolerance, until some evidence of antileukemic activity (eg, fall in peripheral blast count) was noted. Patients were considered to be adequately treated if marrow hypocellularity was achieved or if CNS toxicity appeared without objective response of disease. CNS toxicity was graded as follows: 1+, lethargy; 2+, disorientation with asterixis; and 3+, coma. Responses were assessed as previously described (14).

Enzyme Preparation

The enzyme was prepared according to procedures developed by Roberts et al (9) and was kindly supplied by Kyowa Hakko Kogyo Co, Tokyo, Japan. The enzyme was succinylated as previously described (12,13) and lyophilized. Prior to administration, the enzyme was dissolved in normal saline solution and sterilized by ultrafiltration. Enzyme used in the clinical study had a specific L-glutaminase activity of 200 IU/mg of protein.

RESULTS

Biochemical Determinations

As we noted previously (13), single doses of SAGA did not deplete immediately deproteinized whole

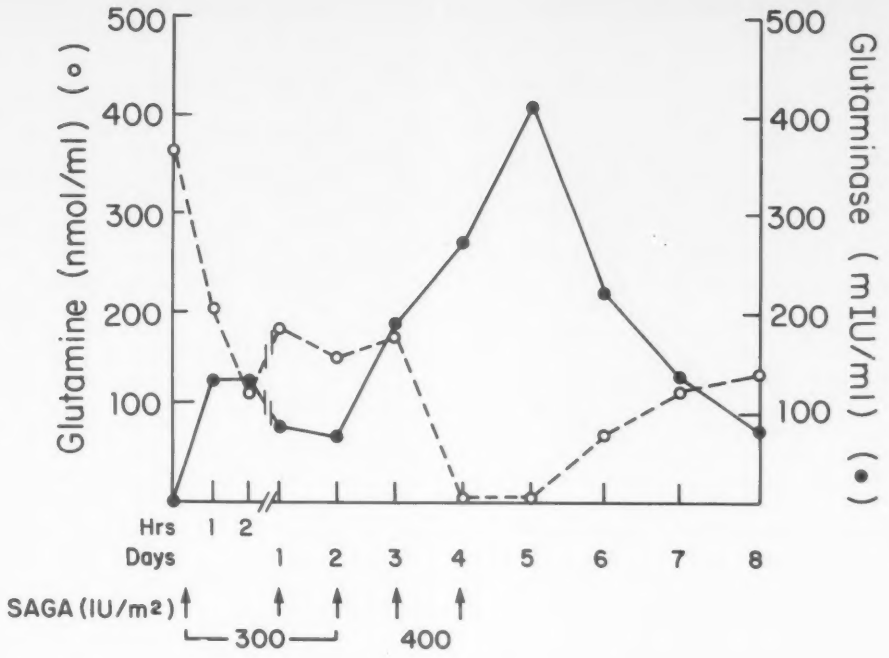

FIGURE 1.—Effect of daily dose administration of SAGA on whole-blood concentrations of glutamine (○) and plasma levels of glutaminase activity (●).

blood of its glutamine content. Several doses administered daily were required before glutamine concentrations fell to consistently undetectable levels (< 5 μM) (fig 1). During SAGA treatment, concentrations of blood glutamate continued to rise, indicating ongoing hydrolysis of glutamine entering from extravascular sources. Ammonia levels rose initially and then appeared to reach a plateau concentration (fig 2).

Clinical Responses

Eleven adult patients with leukemia were entered in this study; two additional patients who received SAGA during the phase I trial at constant daily doses of 320 and 500 IU/m², respectively, are also included in this analysis. Patient diagnoses and clinical responses are presented in table 1.

No patient achieved complete or partial remission. SAGA reduced circulating blast cells in two patients with ALL and in two with CML in blast phase; however, the leukemic cells from both CML patients expressed a lymphoid phenotype (ie, lymphoblastic morphology with elevated terminal deoxynucleotidyl transferase activity) (15). No patient with acute nonlymphoblastic leukemia showed evidence of an antileukemic effect.

One of the patients with blast phase CML achieved a reduction in bone marrow blast cells from > 90% to 20%; no marrow effects were noted in other patients. We also noted a dose-response relationship in this patient (fig 3). Whereas daily administration of SAGA at doses ≤ 400 IU/m² produced no antileukemic effect, dose escalation to ≥ 500 IU/m² caused a pronounced reduction of circulating blast cells which persisted for several weeks after therapy was discontinued.

Toxicity

As expected, CNS toxicity was dose-limiting. Contrary to our expectations from the phase I study, this effect proved clinically unmanageable and prompted early discontinuation of the study. Of the 13 patients treated, five became comatose; three additional patients developed a marked encephalopathy. These CNS effects cleared in all patients within 72 hours of halting enzyme therapy. One patient experienced a fatal intracranial hemorrhage as a result of trauma sustained while he was encepha-

TABLE 1.—Clinical effects of SAGA in patients with leukemia

Patient No.	Diagnosis*	Duration of therapy (days)	Total dose (IU/m^2)	CNS toxicity	Wbc response†
1	CML-Bl	13	6300	3+	↓
2	AMMoL	5	2600	3+	↑
3	ALL	5	1700	3+	0
4	AML	3	1500	3+	0
5	ALL	4	1300	3+	↓
6	AML	4	1300	2+	0
7	AML	4	1300	2+	0
8	ALL	10	4200	1+	0
9	ALL	10	3200	1+	↓
10	CML-Bl	10	3200	1+	↓
11	AMMoL	7	2100	1+	↑
12	ALL	5	1700	1+	0
13	CML-Bl	3	900	2+	↑

*CML-Bl = chronic myelocytic leukemia in blast phase; AMMoL = acute myelogenous, myelocytic leukemia; and AML = acute myelogenous leukemia.

†Wbc = total peripheral blood wbc count. An increase (↑) is ≥ 30% increment over initial value; a decrease (↓) is ≥ 1 log reduction from initial value. Intermediate changes are recorded as 0.

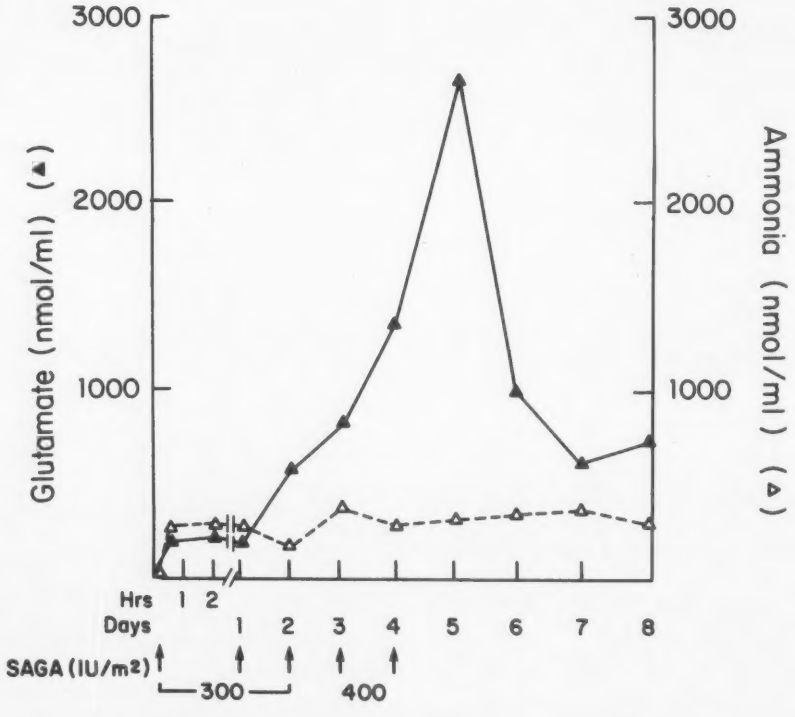

FIGURE 2.—Whole-blood concentrations of ammonia (△) and glutamate (▲) following daily dose administration of SAGA.

lopathic; this death was considered to be drug-related.

Respiratory alkalosis from hyperventilation, probably induced by the elevation in blood ammonia, was noted in all patients. The patient described in figure 3 experienced a gradual decline in serum bicarbonate concentration to 10 mEq/liter; on Day 18 he developed acute, severe hyperglycemia which produced coma, renal failure, and disseminated intravascular coagulation from which he eventually recovered. It is likely that the severity and abrupt onset of this disorder was secondary to exhaustion of buffering capacity stemming from renal bicarbonate loss as compensation for the primary respiratory alkalosis. No disorders of the blood coagulation system were observed in other patients.

Other toxic effects included nausea and vomiting, which consistently occurred at doses $\geq$ 500 IU/m^2. No effect on serum levels of calcium or albumin was noted; no patient developed clinical pancreatitis or hyperamylasemia. Contrary to a previous clinical report of a similar enzyme (16), none of our patients experienced hypersensitivity reactions to this enzyme.

DISCUSSION

SAGA produced excessive CNS toxicity when administered on the dose schedule described. The incidence of substantially greater toxicity in this trial at a dose lower than that administered in a pediatric trial (17) suggests that adults may be more susceptible to these CNS effects. As in hepatic encephalopathy, these toxic effects may be caused by sustained elevations of ammonia concentration in blood and cerebrospinal fluid (13,18), which result from the enzymatic deamidization of both glutamine and asparagine. Because of this toxicity, we cannot draw confident conclusions regarding the theoretic advantages of SAGA relative to *E. coli* asparaginase (ie, broader activity and lack of induced resistance).

Although only patients with lymphoblastic phenotype showed clinical response, therapy with higher doses of enzyme for more prolonged duration might produce responses in patients with acute nonlymphoblastic leukemia, provided that amelioration of the CNS effects could be achieved. Such responses have been reported in clinical trials with similar glutaminase-asparaginase enzymes (19).

It is conceivable that an alternative dose schedule might increase the therapeutic index for this drug. Daily administration was selected in view of the high incidence of hypersensitivity reactions seen with intermittent enzyme dosage (2,20) and because of pharmacologic data obtained during our phase I evaluation. Previous studies have reported quite

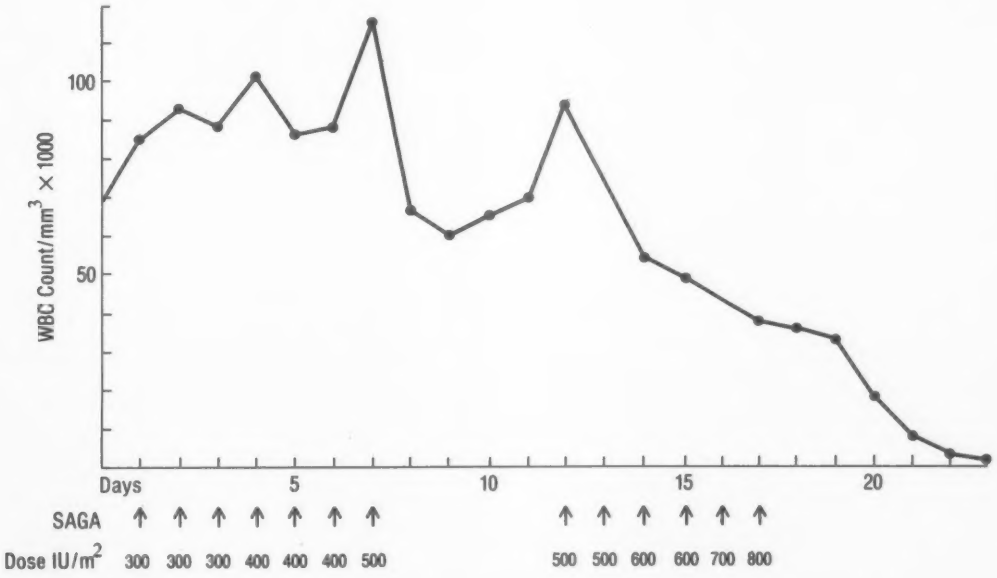

FIGURE 3.—Dose-response relationship between increasing doses of SAGA and peripheral wbc count in a patient with blast phase CML.

prolonged depletion of plasma glutamine (for several days) following single doses of glutaminase-asparaginase (19,21). We found, however, that single doses of SAGA only transiently lowered blood glutamine to nondetectable levels when the analysis was performed on blood in which the enzyme was inactivated by deproteinization at the bedside (13). This method measures total amino acid concentration in whole blood (ie, that contained in both the plasma and intracellular compartments). However, the failure to achieve prolonged glutamine depletion in whole blood following enzyme administration cannot be ascribed to the persistence of an intraerythrocytic "glutamine reservoir." Using this method, we have shown that whole-blood glutamine levels *are* depleted by single doses of enzyme; however, these levels rapidly recover (13). We again noted that serial daily doses of SAGA were required to achieve and maintain continuous glutamine depletion in whole blood (fig 1). Since the enzyme appears to be confined to intravascular volume (13), it seems likely that low levels of circulating glutaminase in blood which is not immediately deproteinized will hydrolyze glutamine ex vivo, whereas actual in vivo blood glutamine concentrations are higher as a result of continuous glutamine influx from extravascular sources. It is not clear, however, what level of blood (or tissue) glutamine depletion is necessary to realize antitumor effects. While we noted a definite dose-response relation to SAGA in one of our patients (fig 3), Tallal et al (22) reported that daily doses of asparaginase which ranged from 10 to 5000 IU/kg all produced equivalent rates of complete remission in childhood leukemia. Moreover, blood asparagine levels were rarely measured during the clinical trials of asparaginase. Therefore, the necessity of achieving prolonged depletion of amino acids in blood remains uncertain.

Given the toxicity of daily administration, an alternative schedule employing short daily courses of enzyme in combination with an antimetabolite or an amino acid analog might be of greater clinical utility. The combination of the glutamine analogs DON (6-diazo-5-oxo-L-norleucine) or acivicin with glutaminase-asparaginase achieves substantially greater antitumor effects than any of these agents alone (23,24). Furthermore, the concurrent use of these analogs with glutaminase-asparaginase appears to prevent the formation of antibodies to the enzyme in experimental animals (24). Thus, the clinical use of such combinations might achieve the desired antineoplastic effects while minimizing enzyme toxicity.

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The Course of Nausea and Vomiting After High-Dose Cyclophosphamide[1,2]

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SUMMARY

We studied the course of nausea and vomiting after high-dose cyclophosphamide (50–75 mg/kg) in 25 patients. Nausea was assessed with a visual analog scale and vomiting by patient report. The reliability and validity of these methods of assessment were established in separate studies of oncology patients. Nausea and vomiting developed between 6 and 12 hours after the start of a 1-hour cyclophosphamide infusion in about two thirds of the patients. Peak symptoms were at 12 hours. Vomiting had subsided in most of the patients by 24 hours, but nausea persisted. Although there was significant individual variation in the course of nausea and vomiting, we could identify no demographic, disease, drug, or treatment factors which were associated with more significant nausea and vomiting. Some of the variability in the onset, severity, and duration of nausea and vomiting may be related to individual differences in the metabolism of and susceptibility to cyclophosphamide. The visual analog scale may provide information about nausea not obtained by the more frequently employed categoric verbal rating scales. Suggestions for the treatment of nausea and vomiting after high-dose cyclophosphamide are made on the basis of the findings.

[Cancer Treat Rep 66:1487–1493, 1982]

Cyclophosphamide is a frequently prescribed antineoplastic drug. Large doses (≥ 50 mg/kg) have been employed in the treatment of small cell carcinoma of the lung (1) and multiple myeloma (2) and in preparation for bone marrow transplantation (3–5). Unfortunately, such large doses cause severe vomiting (6–8). However, there are no careful descriptions in the literature of the course of nausea and vomiting after cyclophosphamide treatment. Such a description may improve our understanding of the toxicology of cyclophosphamide emesis and suggest better antiemetic treatment plans. We report on the nausea and vomiting caused by high-dose cyclophosphamide in 25 patients who were studied by reliable and valid methods for the measurement of nausea and vomiting.

METHODS

Sample

Table 1 provides data that describe our study group. Twenty-five hospitalized patients (20 males and five females) with a mean age of 42 years (range,

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TABLE 1.—Characteristics of 25 patients who received high-dose cyclophosphamide

Patient No.	Age (yrs)	Sex	Disease*	Cyclophosphamide dose (mg/kg)	Antiemetics	Allopurinol	Emesis from previous chemotherapy
1	47	M	HCL	75	Prochlorperazine	Yes	No
2	21	M	AA-BMT	50	Prochlorperazine	No	No
3	64	M	MM	60	Prochlorperazine	Yes	Yes
4	40	M	CML	75	Antihistamines	Yes	No
5	19	M	ANLL	75	Fluphenazine, chlorpromazine	Yes	Yes
6	19	F	ALL	75	Prochlorperazine	No	No
7	20	M	ALL-BMT	50	Prochlorperazine	Yes	Yes
8	55	F	SCCL	75	Prochlorperazine	No	No
9	45	M	AA-BMT	50	Chlorpromazine	No	No
10	21	M	ALL	75	Prochlorperazine	Yes	Yes
11	63	M	MM	60	Prochlorperazine	Yes	Yes
12	31	M	HCL-BMT	50	Antihistamines	Yes	No
13	71	M	MM	60	Prochlorperazine	Yes	Yes
14	33	M	HCL-BMT	50	Antihistamines	Yes	Yes
15	52	M	ANLL	75	Prochlorperazine	Yes	Yes
16	62	M	SCCL	75	Prochlorperazine	Yes	No
17	48	F	AA-BMT	50	Prochlorperazine	No	No
18	27	M	AA-BMT	50	Prochlorperazine	No	No
19	54	M	CML	75	Antihistamines, prochlorperazine	Yes	No
20	24	M	AA-BMT	50	Phenobarbital	No	Yes
21	52	M	SCCL	75	Prochlorperazine	No	No
22	51	M	SCCL	75	Prochlorperazine	Yes	No
23	59	F	MM	60	Chlorpromazine, prochlorperazine	Yes	No
24	42	M	ANLL	75	Antihistamines	Yes	Yes
25	32	F	MM	50	Chlorpromazine	Yes	Yes
N = 25	Mean, 42.1	20 M 5 F		Mean, 63.6		Yes = 17; No = 8	Yes = 11; No = 14

*AA = aplastic anemia, ALL = acute lymphocytic leukemia, ANLL = acute nonlymphocytic leukemia, CML = chronic myelogenous leukemia, HCL = hairy cell leukemia, MM = multiple myeloma, SCCL = small cell carcinoma of the lung, and BMT = bone marrow transplant.

19–71) participated in the study. Cyclophosphamide (50–75 mg/kg) was given as a 1-hour iv infusion to 21 patients, as a 1–2-hour infusion to three patients, and as a 4-hour infusion to one patient. Nine patients received 50 mg/kg, four received 60 mg/kg, and 12 received 75 mg/kg. The mean dose of cyclophosphamide was 63.6 mg/kg. Patients received no other chemotherapeutic agent in the week before or 24 hours after the administration of cyclophosphamide. Cyclophosphamide was administered for small cell carcinoma of the lung (four patients), multiple myeloma (five), acute leukemia (five), chronic leukemia (two), and in preparation for bone marrow transplantation for a variety of diseases (nine). The patients were ambulatory and able to care for themselves (Eastern Cooperative Oncology Group performance status 0 or 1). They were ineligible for the study if they had any condition or were receiving any medication which regularly causes vomiting. Eleven patients had received emesis-producing chemotherapy previously, and of these, four had received high-dose cyclophosphamide. Various antiemetic therapies were employed. Fifteen patients received prochlorperazine alone, one received prochlorperazine combined with antihistamine antiemetics, two were treated with chlorpromazine alone,

one received chlorpromazine plus fluphenazine, four were treated with antihistamines, and one was treated with phenobarbital. Twelve of the 15 patients who received prochlorperazine alone were part of a pilot study to see if ≥ 12 hours of pretreatment with 6-hourly doses of 5–10 mg of prochlorperazine was a promising antiemetic approach. The remaining 13 patients had their antiemetic treatment initiated at the time their chemotherapy was given or when they developed nausea and vomiting. No patients received cannabinoids as antiemetics. Seventeen patients were receiving allopurinol at the time they received cyclophosphamide. No other medication was prescribed with significant frequency. Patients were allowed food, fluids, and activity ad libitum after receiving cyclophosphamide.

Nausea and Vomiting Assessment

Nausea and vomiting were assessed by self-report. The first assessment was completed just prior to the cyclophosphamide infusion and then every 3 hours for 24 hours. Nausea was evaluated using a visual analog scale bounded on one end by "not at all nauseous" and at the other end by "extremely nauseous." Patients indicated the nausea they were experiencing at the time they completed the scale by making a slash mark at an appropriate place on the 100-mm line. The nausea score was the number of millimeters from the "not at all nauseous" end of the line to the patient's mark. At each assessment, patients also reported the number of times they had vomited in the preceding 3-hour period. Vomiting was defined as the expulsion of gastric contents.

Although the reliability and validity of the analog method are established for a variety of conditions (9), these studies were repeated for nausea in separate studies of oncology patients. Reliability of the visual analog scale as a method for measuring nausea was assessed by test-retest. Patients were asked to mark the line and then were asked to mark it again 10–15 minutes later. Eighteen patients (11 males and seven females; mean age, 43 years) provided 27 pairs of nausea test-retest scores over a wide range of nausea scores for the reliability study.

The validity of the analog method for measuring nausea was established by comparison of the patient's nausea score with the assessment of a single clinical observer (J. H. Fetting) who scored the patient as nauseous or not after a brief clinical interview. The observer was unaware of the patient's self-report nausea score.

We established a cut-off score which indicated nausea. A random sample of 26 oncology patients (14 males and 12 females; mean age, 46 years) who stated they were not nauseous were asked to mark the analog scale. The mean score in this group was 10, with a standard deviation of 12. A score of 22 (one standard deviation above the mean) was then arbitrarily set as representing a "case" of nausea by self-report.

Twenty randomly selected oncology patients (13 males and seven females; mean age, 41 years) comprised the sample for the validation trial. Two-by-two tables were used to examine the relationship between self-report scores above and below 22 and the assessment of the clinical observer.

The self-report of vomiting was also validated by comparison with the report of a blinded clinical observer. The same sample of 20 patients used for the nausea validation trial was employed. A two-by-two table was employed to compare the patient's self-report of vomiting with that of the clinical observer.

RESULTS

Reliability and Validity of Self-Report Assessment

The coefficient of correlation between the 27 test-retest nausea scores was 0.83. This was judged to be an acceptable level of reliability. In the validity trial, a self-report score of 22 was used for comparison with the report of the clinical observer. A two-by-two table was employed to analyze the relationship between self-report nausea scores and the report of the clinical observer. With a score of ≥ 22 indicating nausea, there was a significant relationship between the patient report and that of the clinical observer ($P < 0.025$ by chi-square test). The relationship between self-report of vomiting and the report of the observer was significant at the $P < 0.001$ level by chi-square test.

Nausea and Vomiting After High-Dose Cyclophosphamide

Twenty-three of 25 (92%) patients experienced nausea (analog scale score ≥ 22) at some time during the 24 hours after cyclophosphamide. Twenty-three (92%) patients vomited, including one of the two patients who was not nauseous at any 3-hour assessment. One patient had nausea and one episode of vomiting just prior to the cyclophosphamide infusion. He attributed that to 5 mg of iv prochlorperazine. This medication was discontinued and his nausea and vomiting subsided over the next 6 hours. Both nausea and vomiting increased dramatically between 6 and 9 hours after cyclophosphamide, presumably in response to cyclophosphamide.

Figure 1 shows the number of patients developing nausea over time. Twenty-two of 23 (96%) patients developed nausea within 12 hours of drug administration, 15 of 23 (65%) first developed nausea between 6 and 12 hours after cyclophosphamide, and seven of 23 (30%) did so within the first 6 hours. The patient who first reported nausea at 18 hours had reported vomiting at several previous assessments, suggesting that his nausea may have been intermittent. Figure 2 shows the mean nausea scores of the sample at each assessment time; it also demonstrates that nausea increases dramatically between 6 and 12 hours after cyclophosphamide. Although the peak nausea score was at 12 hours, there was no statistically significant difference (by t test) among the scores from 9 to 21 hours. Thirteen of 25 (52%) patients reported their peak nausea score at 12 hours. The next most frequent peak nausea time was at 15 hours at 16% of the patients. Fifty-four percent of the patients were still nauseous 24 hours after cyclophosphamide.

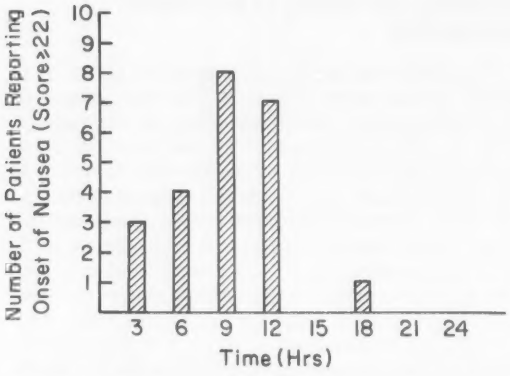

FIGURE 1.—Development of nausea over time after high-dose cyclophosphamide. Cyclophosphamide was infused over the 1st hr in 84% of the patients.

Figure 3 shows the development of vomiting in our patients over time. Vomiting tended to have a somewhat later onset than nausea. Sixteen of 23 (70%) patients developed vomiting between 6 and 12 hours after cyclophosphamide but only three of 23 (13%) developed vomiting in the first 6 hours. Four of 23 (17%) patients developed vomiting > 12 hours after cyclophosphamide. Figure 4 shows the mean number of vomiting episodes during the previous 3 hours reported at each assessment time. Vomiting increased dramatically between 6 and 12 hours after cyclophosphamide. Peak vomiting was reported at 12 hours but there was no statistically

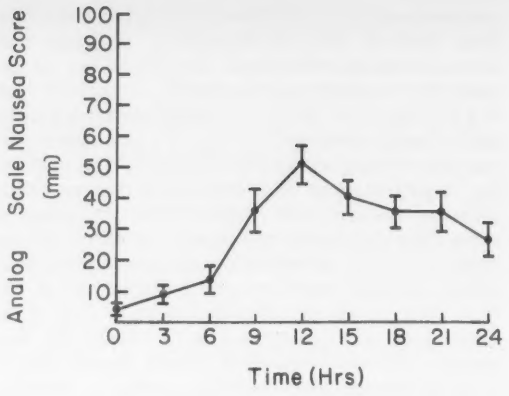

FIGURE 2.—Mean nausea scores of patients (± SE) over time after high-dose cyclophosphamide.

significant difference (by t test) between the number of vomiting episodes reported at 12 hours and those reported at 9, 15, and 18 hours. Vomiting had significantly declined from the 12-hour peak at 21 hours, and by 24 hours the mean value was less than one episode. Only 33% of the patients reported vomiting between 21 and 24 hours after cyclophosphamide. Patients reported vomiting an average of 8.6 times during the 24 hours after cyclophosphamide.

As can be seen from the preceding data, there is significant individual variation in the onset, sever-

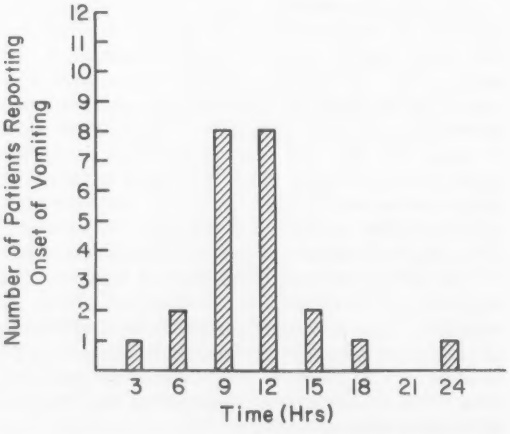

FIGURE 3.—Development of vomiting over time after high-dose cyclophosphamide. Cyclophosphamide was infused over the 1st hr in 84% of the patients.

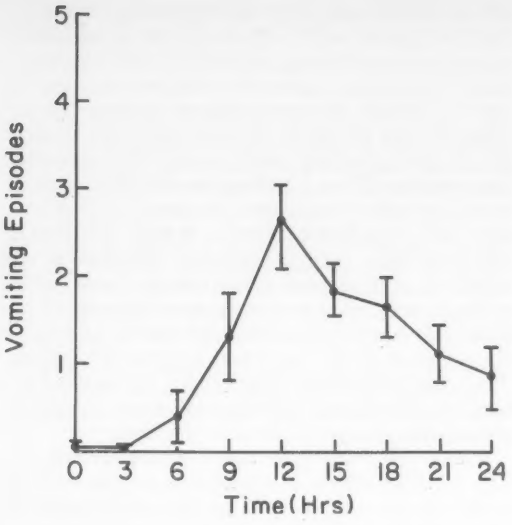

FIGURE 4.—Mean No. of vomiting episodes (± SE), reported by patients during the previous 3 hrs, over time after high-dose cyclophosphamide. Cyclophosphamide was infused over the 1st hr in 84% of the patients.

ity, and duration of nausea and vomiting after high-dose cyclophosphamide.

We attempted to identify factors in subsets of our patients which were associated with more severe nausea and vomiting. We subdivided our patients according to age (above and below the mean age of 42 years), sex, disease, and cyclophosphamide dose (50 and 60 vs 75 mg/kg). In addition, we contrasted patients who had been treated with and without allopurinol and prochlorperazine pretreatment. Finally, we compared patients who had previously received emesis-producing chemotherapy with those who had not. For each of these subgroups we compared mean values (by t test) for onset, duration, and severity of nausea and vomiting. Dividing our sample in these ways did not reveal any subgroups with a significantly different course of nausea and vomiting after high-dose cyclophosphamide.

DISCUSSION

We found that nausea and vomiting occurred very predictably after high-dose cyclophosphamide. There was a 6–12-hour latency period from drug administration until the development of nausea and vomiting in 65%–70% of our patients. Peak symptoms occurred 12 hours after cyclophosphamide but the entire period from 9 to 18 hours was marked by severe nausea and vomiting. Although vomiting had decreased significantly by 24 hours after cyclophosphamide, most of the patients were still nauseous. Since 84% of our patients received cyclophosphamide as a 1-hour infusion, we were unable to examine the relationship between rate of cyclophosphamide administration and the course of nausea and vomiting. A 6-hour delay in the onset of nausea and vomiting after cyclophosphamide has been commented on previously. It was suggested that this delay was related to the time required for the hepatic metabolism of the drug to cytotoxic and presumably emetic metabolites (10).

We could not demonstrate a dose-response relationship between cyclophosphamide dose and nausea and vomiting over the range of 50–75 mg/kg. Although this may be related to small patient size, it is also possible that at such large doses nausea and vomiting are at maximal levels. If this were the case, nausea and vomiting after more modest doses of cyclophosphamide might demonstrate a more typical dose-response relationship. An inverse relationship has been noted between mechlorethamine dose and vomiting severity and latency in cats (11).

We were unable to identify other factors which contributed to the individual variation we observed in the course of nausea and vomiting. There was no apparent relationship between age, sex, or underlying disease and the course of nausea and vomiting. Since the number of female patients and the number of patients with each underlying disease were small, it is possible that true differences could have been missed. We could not demonstrate a systematic effect of allopurinol on nausea and vomiting. Allopurinol competes with cyclophosphamide for hepatic microsomal enzymes but has not been found to appreciably alter cyclophosphamide's cytotoxic effects (12). Since 92% of our patients received phenothiazine antiemetics, it is impossible to discern an effect of this class of drugs on the course of nausea and vomiting. On the other hand, this high percent receiving phenothiazines provided for antiemetic homogeneity in our patients. It would be more difficult to analyze the course of nausea and vomiting after cyclophosphamide in patients receiving many different antiemetics. Since phenothiazines are supposedly effective antiemetics for post-chemotherapy vomiting (13), the duration and severity of nausea and vomiting observed after cyclophosphamide may underestimate the true values. Since nausea and vomiting remained severe in these patients despite phenothiazine antiemetics, we believe antiemetic treatment had a relatively minor impact on the time course of nausea and vomiting. The use of more effective antiemetics, how-

ever, might change the time course as well as the severity of nausea and vomiting after high-dose cyclophosphamide. Although there are reports (14) that patients who have previously received emesis-producing chemotherapy might have a different course of nausea and vomiting than naive patients, we could not demonstrate it in these patients. Although not systematically studied, we asked 20 of our patients to choose from a list of 13 common hospital activities those which palliated and exacerbated their nausea and vomiting. Seventy percent indicated that sleeping palliated nausea and vomiting, 5% felt it exacerbated the syndrome, and 25% had no preference. Eating and drinking exacerbated nausea and vomiting for 55% and palliated for none. Since we did not attempt to control or systematically assess such variables, it is unclear how they may have influenced our results. Control of such variables as oral intake has been attempted in some antiemetic studies (15) but apparently not in others (16,17). Other investigators have written about the effect of activities of daily living on the course of nausea and vomiting after chemotherapy (18). Finally, it is not known what effect individual differences in the metabolism of cyclophosphamide might have on the course of nausea and vomiting.

We employed a reliable, valid, visual analog scale to measure nausea. By deriving a cut-off score for nausea we were also able to divide our patients categorically according to the presence or absence of nausea. The categoric analysis was more useful for determining the onset and duration of nausea, whereas the analog scale was most helpful for following the severity of nausea over time, especially in an individual patient. The visual analog scale has rarely been employed in the measurement of nausea associated with chemotherapy or in antiemetic studies (19). Most such studies use a categoric verbal rating scale (15–17). The visual analog has been widely used in the assessment of pain, mood, appetite, etc (9,20–22). There are reports that the visual analog scale is more sensitive than a categoric scale for measuring pain (23,24). It seems possible that the visual analog scale could contribute increased sensitivity to the measurement of nausea as well. Self-report is an inexact method for monitoring emesis. Since vomiting is an observable behavior involving many physiologic changes, some direct measurement system could be devised for monitoring it. Preliminary studies of respiratory inductance plethysmography for monitoring cat vomiting suggest that this method may be applicable to the study of emesis in man (25).

Based on this study we can suggest some modifications in the antiemetic strategies for high-dose cyclophosphamide. Antiemetics are usually given on a fixed-dose schedule. Since the nausea and vomiting associated with high-dose cyclophosphamide varied in intensity over the 24-hour observation period, a fixed-dose antiemetic schedule may not be optimal. It may be worthwhile to increase the antiemetic dose or dose interval to cover the peak in nausea and vomiting which occurs 12 hours after the initiation of the cyclophosphamide infusion. Since more aggressive antiemetic treatment at this time may cause significant sedation, it may be useful to start high-dose cyclophosphamide infusions in the afternoon so that peak symptoms and antiemetic-induced sedation occur during usual sleeping hours. Since there may be an increased risk of aspiration pneumonia with this approach, appropriate precautions should be taken. Additionally, the sedated patient must be awakened every 1–2 hours to urinate if hemorrhagic cystitis is to be avoided.

Antiemetic therapy for many anticancer drugs is only partially effective. Careful descriptions of the course of emesis after these agents will improve our understanding of their toxicology and allow us to devise more specific antiemetic strategies.

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Essential Thrombocythemia: Response During First Year of Therapy With Melphalan and Radioactive Phosphorus: A Polycythemia Vera Study Group Report[1,2]

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SUMMARY

Thirty-one patients with essential thrombocythemia were randomized to receive either melphalan or radioactive phosphorus as myelosuppressive therapy. Twenty-seven patients were evaluable for response. Of 13 patients treated with melphalan, 11 had a complete response (platelet count < 450,000/mm^3) at 3 and 6 months. This response rate was significantly better than the response to radioactive phosphorus. The response rates were similar at 12 months. No significant toxicity was observed with either regimen.

[Cancer Treat Rep 66:1495–1500, 1982]

Thrombocytosis is a frequent manifestation of all the myeloproliferative disorders (MPD) (1). There are reports of patients who tolerate strikingly high platelet levels for many years without clinical sequelae (2). However, several reports (3–6) involving small numbers of patients suggest that such patients are at increased risk for thrombotic and hemorrhagic complications and that such complications can be reduced in frequency and severity by lowering the platelet count with myelosuppressive therapy. Within the large spectrum of MPD, there is a subgroup of patients who have thrombocytosis without accompanying manifestations of polycythemia vera, chronic granulocytic leukemia, or myelofibrosis (agnogenic myeloid metaplasia). This subgroup has been labeled essential or primary thrombocythemia (ET). Again, despite reports of small series, there are no prospective studies of a large group of ET patients which would define their response to myelosuppressive therapy. This report describes the experience of the Polycythemia Vera Study Group (PVSG) during the first year of treatment of ET with melphalan (Alkeran) and radioactive phosphorus (^{32}P).

MATERIALS AND METHODS

Between 1975 and 1980, the PVSG conducted two studies, PVSG 04 and PVSG 10, for the registration and study of patients with thrombocytosis secondary to MPD (7). Both protocols were identical except that in the second study, PVSG 10, patients < 40 years of age were excluded and the dose of ^{32}P was increased (*see* below). Patients were randomized in blocks of four within each country of origin to receive either melphalan or ^{32}P. The initial dose of melphalan was 10 mg/day for 5 successive days. The dose was then reduced to 2 mg/day for the following 3 weeks. The subsequent dose of melphalan was adjusted for continuous maintenance of the platelet count in the range of 200,000–400,000/mm^3. In PVSG 04, the initial dose of ^{32}P was 2.3 millicuries/m^2 iv, not to exceed 5.0 millicuries/dose. During the course of this protocol, it was recognized that this dose of ^{32}P was not consistently effective

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in producing a prompt reduction in the platelet count. Therefore, in PVSG 10, the initial dose was increased to 2.9 millicuries/m^2, not to exceed 5 millicuries/dose. ^{32}P was administered at intervals of 3 months, if needed, to maintain the platelet count < 450,000/mm^3. Response to therapy was evaluated at 3, 6, and 12 months by recording the lowest platelet count in three time intervals after start of therapy: 9–17, 20–32, and 44–60 weeks. The patient was listed as having a complete response (CR) if that platelet count was < 450,000/mm^3. If that platelet count was in the range of 450,000–600,000/mm^3, 600,000–1,000,000/mm^3, or > 1,000,000/mm^3, the patient was listed as having a good partial response (PR), a poor PR, or no response, respectively. Crossover to the alternate therapy arm was permitted at 6 months if there was no response to the primary treatment.

Among patients entered and randomized over a 5-year period, 31 were subsequently identified who met the following strict criteria for the diagnosis of ET: (*a*) Platelet count ≥ 1,000,000/μl on at least two separate determinations. (*b*) Absence of the Philadelphia chromosome in metaphases obtained from marrow aspirates. (*c*) Normal rbc mass measured with ^{51}chromium-labeled rbcs (8) (this requirement was waived if the hemoglobin was < 13 g/100 ml; four patients). (*d*) Iron present on iron stain of marrow (if absent, a patient was considered eligible if a trial of oral iron therapy for 1 month resulted in no increase in hemoglobin; three patients). (*e*) No fibrosis on marrow biopsy (if fibrosis was present, a patient was considered eligible if the fibrosis was less than one third of the marrow sectional area and was not associated with splenomegaly and leukoerythroblastic blood picture; one patient). (*f*) Thrombocytosis could not be attributed to some other identifiable cause, ie, infection, chronic inflammatory disease, malignancy, previous splenectomy, etc.

Within each study, characteristics of patients on the two treatment regimens were compared using methods of descriptive statistics. Similarly, response rates in the two treatments were compared, adjusting for protocol using the Mantel-Haenszel

TABLE 1.—Initial characteristics of patients by protocol and treatment group

	No. of patients				
	PVSG 04		PVSG 10		
Characteristic	^{32}P	Melphalan	^{32}P	Melphalan	Total
No. in group	8	7	9	7	31
Age (yrs) at entry					
< 50	1	2	2	0	5
50–70	4	2	5	4	15
> 70	3	3	2	3	11
Sex					
Male	5	4	3	5	17
Female	3	3	6	2	14
Spleen					
Enlarged	3	2	2	2	9
Not enlarged	5	5	7	5	22
Liver					
Enlarged	2	0	1	0	3
Not enlarged	6	7	8	7	28
Platelet count (cells × 10^3/mm^3)					
< 1000	0	1	0	0	1
1000–1499	6	4	5	5	20
1500–2000	1	1	3	1	6
> 2000	1	1	1	1	4
Wbc count (cells × 10^3/mm^3)					
4.5–12	5	4	4	4	17
> 12	3	3	5	3	14
Hgb (g/100 ml)					
9–11.9	3	2	2	2	9
12–15	4	4	6	2	16
> 15	1	1	1	3	6

TABLE 2.—Response to therapy by treatment and protocol (evaluable patients)

	No. of patients (%)			
	PVSG 04		PVSG 10	
	^{32}P	Melphalan	^{32}P	Melphalan
3-mo response				
CR	1 (17)	4 (67)	5 (63)	7 (100)
Good PR	1 (17)	1 (17)	1 (13)	0 (0)
Poor PR	1 (17)	1 (17)	2 (25)	0 (0)
No response	3 (50)	0 (0)	0 (0)	0 (0)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)
Totals	6 (100)	6 (100)	8 (100)	7 (100)
6-mo response				
CR	1 (17)	4 (67)	5 (63)	7 (100)
Good PR	2 (33)	2 (33)	1 (13)	0 (0)
Poor PR	2 (33)	0 (0)	1 (13)	0 (0)
No response	1 (17)	0 (0)	1 (13)	0 (0)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)
Totals	6 (100)	6 (100)	8 (100)	7 (100)
12-mo response				
CR	3* (50)	3 (50)	5 (63)	5 (71)
Good PR	0 (0)	2 (33)	3 (37)	2 (29)
Poor PR	3 (50)	0 (0)	0 (0)	0 (0)
No response	0 (0)	0 (0)	0 (0)	0 (0)
Unknown	0 (0)	1 (17)	0 (0)	0 (0)
Totals	6 (100)	6 (100)	8 (100)	7 (100)

*1 patient had CR at 12 mos after switching to melphalan at 6 mos. He had had no response to ^{32}P at 6 mos.

chi-square test. Four of the 31 patients identified with ET were ineligible for the evaluation of therapy for the following reasons: one PVSG 04 patient randomized to receive melphalan was lost to followup, and three patients randomized to ^{32}P (two in PVSG 04 and one in PVSG 10) were treated with only 1.5–1.7 millicuries/m^2, less than the prescribed initial dose. Of the 27 patients eligible for evaluation, 12 were randomized in PVSG 04, six in each treatment group. Of the 15 eligible patients randomized in PVSG 10, seven were treated with melphalan and eight with ^{32}P.

Characteristics of these ET patients at time of randomization to each study (PVSG 04 and PVSG 10) are shown by treatment group in table 1. In general, within each study, patients in the two treatment groups are comparable. Fifty-five percent of these patients were male; 84% were > 50 years old. No patients < 50 years of age were randomized to melphalan in PVSG 10. Twenty-nine percent of the ET patients had a palpable spleen, but the largest recorded was only 4 cm below the costal margin. Ten percent had a palpable liver which was judged clinically to be enlarged. Mild anemia and leukocytosis were characteristic of a minority of patients. Twenty-nine percent had peripheral hemoglobin < 12 g/100 ml, but none had hemoglobin < 9 g/100 ml. The peripheral wbc count was > 12,000/mm^3 in 45%. The initial platelet count was < 1,000,000/mm^3 in one PVSG 04 patient treated with melphalan; two subsequent measurements, however, were > 1,000,000/mm^3. Of the 31 patients, seven had a history of thrombosis, seven had a history of hemorrhage, two had a history of both, and 15 had a history of neither. Thrombotic manifestations included angina (four patients), venous thrombosis (three), transient ischemic attacks (three), cerebrovascular accident (one), and peripheral arterial disease (one). Hemorrhagic manifestations included epistaxis (five patients), easy bruising (three), postoperative hemorrhage (one), gastrointestinal hemorrhage (one), and retinal hemorrhage (one).

RESULTS

The CR rate at 3 months for PVSG 04 was 17% for ^{32}P compared with 67% for melphalan (table 2). Similarly, for PVSG 10 at 3 months the CR rate of 100% for melphalan was somewhat higher than the 63% CR rate for ^{32}P. In fact, the CR rate is significantly higher for melphalan than for ^{32}P, adjusting for protocol at 3 months (Mantel-Haenszel $\chi^2_1 = 4.0$, corrected for continuity, $P = 0.05$). The CR rates at 6 months similarly favor melphalan. However, at 12 months, the CR rates for the two treatments are comparable, adjusting for protocol.

All patients treated with melphalan achieved a CR at one of the three evaluation intervals during

the 12 months; in fact, all patients except two in PVSG 04 reached CR at 3 months. Only four of the six PVSG 04 patients receiving ^{32}P ever achieved a CR and three[3] of these four patients required 12 months to do so. In PVSG 10, all eight patients treated with ^{32}P achieved a CR at some point. Five of the eight did so within 3 months. Thus, in PVSG 10, the higher ^{32}P dose appeared to be associated with more rapid and complete response.

The immediate toxicity of these two regimens was negligible; no significant leukopenia or thrombocytopenia was reported. Two patients had disease complications during the study period. One patient had a cerebrovascular accident 9 days after the initiation of melphalan therapy when the platelet count was 730,000/mm^3; one patient had a deep vein thrombosis after 6 months of therapy with ^{32}P when the platelet count was 1,350,000/mm^3. There were no hemorrhagic complications.

DISCUSSION

The patients reported on in this study represent the initial experience of the PVSG with therapy for ET. In general, the patient characteristics are consistent with descriptions of small groups of patients in the literature. Splenomegaly, hepatomegaly, mild anemia, and leukocytosis were observed, but only in a minority of patients. Only one third of our patients had platelet counts > 1,500,000/μl and only one half had symptoms of thrombosis or hemorrhage. Many of the small series in the literature described patients with more striking increases of the platelet count and more striking symptoms. It is likely that this reflected the tendency to report dramatic examples of a relatively rare disease. We know of no bias within our institutions which would lead to the exclusion of more severely affected cases.

All patients were treated with myelosuppressive therapy, with a goal of permanently lowering the platelet count to < 450,000/mm^3. This was easily and safely accomplished using melphalan, confirming the report of Bensinger et al (6) in a smaller series. The response rate to ^{32}P was significantly slower. The CR rate at 3 months was less than that observed with melphalan, and six of the 14 patients treated with ^{32}P had a platelet count > 600,000/mm^3 at that point. This result may be partially explained by dose since the results were somewhat better when the ^{32}P dose was raised from 2.3 to 2.9 millicuries/m^2 in PVSG 10. Either drug was tolerated during the study period without significant toxicity.

These studies do not address two important questions. The first is whether patients without symptoms of thrombosis or hemorrhage need to be treated. The second is whether patients who have achieved a good remission with a course of therapy need to continue in a maintenance program which maintains a normal platelet count. Our results show that remission and maintenance can be accomplished easily. However, both of the agents employed will almost certainly increase the risk of second malignancies, particularly if they are employed in a maintenance program (9,10). The data presented here are limited to experience with remission induction and the first year of management. The patients continue under observation; their long-term followup will be recorded in a future report.

[3]Includes one patient in PVSG 04 treated initially with ^{32}P who had a 12-month CR after switching to melphalan at 6 months. He had had no response to ^{32}P at 6 months.

APPENDIX

The collaboration of the members of the Polycythemia Vera Study Group who are listed below is acknowledged. Without their contributions to the study, the results mentioned in all papers would not have been possible.

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Management of Pelvic Complications of Malignant Urothelial Tumors With Combined Intra-Arterial and Iv Chemotherapy[1]

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SUMMARY

Twenty-nine patients with local-regional pelvic recurrences of malignant urothelial tumors were treated with combined intra-arterial and iv chemotherapy. 5-FU (1 g/m²) was infused daily for 5 days via bilateral percutaneously placed hypogastric catheters. Doxorubicin (25 mg/m²) was infused iv on Days 1 and 2 and mitomycin (5 mg/m²) was infused iv on Days 3 and 4 of each course. Seventeen patients (58%) demonstrated an objective decrease in the size of the pelvic mass. All four patients with one of the adenocarcinoma variants of malignant urothelial tumors responded to therapy, while the three patients with squamous transformation of a transitional cell carcinoma failed to respond. The median survival of the responding patients was significantly longer (52 weeks) than that of nonresponding patients (28 weeks) ($P = 0.002$). Patients with initial low-volume pelvic disease demonstrated durable control of local residual tumors, with four of six patients having negative repeat cystoscopic examinations.

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The treatment of patients with locally advanced bladder cancer is unsatisfactory. Many modalities have been introduced for the palliation of pelvic symptoms, with only modest success (1). Attempts with intra-arterial chemotherapy infused via bilateral hypogastric artery catheters have had encouraging results. 5-FU and mitomycin have each been extensively studied and have achieved consistent objective response (2,3).

The advantage of intra-arterial over iv chemotherapy can be theoretically attributed to the higher concentration of drug delivered to the infused site. The drug concentration achieved in the target site is inversely proportional to the flow rate of the infused vessel and directly proportional to the total quantity of drug administered. Systemic toxicity is determined by the rate of drug elimination, while local toxicity is determined by the drug concentration in the tissue (4). The hypogastric vessels offer a readily approachable vascular system with a relatively low flow rate. 5-FU is an ideal agent for arterial infusion because of the rapid drug elimination and modest bone marrow toxicity when it is delivered as a continuous infusion (5,6).

In an attempt to increase the rate and duration of previously described objective responses in the management of pelvic recurrences of malignant urothelial tumors, we have adopted 5-FU as the single agent to be administered intra-arterially at maximum tolerated dosages. To this we have added the simultaneous iv infusion of doxorubicin and mitomycin. These two agents were selected because of their previously described activity against malignant urothelial tumors (7,8) and their clinically documented synergism (9).

The 29 patients treated were selected because each had as a dominant clinical problem the pres-

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ence of threatening local pelvic complications of the tumor. To distribute the drug more accurately in each catheter, we employed radionuclide angiography. We believe this technique reflects more faithfully the distribution of the infused drug and allows for the estimation of the proportional infusion of each catheter to the tumor bed.

MATERIALS AND METHODS

Twenty-nine patients with predominant pelvic complications of malignant urothelial tumors were treated with a combination of intra-arterial and systemic chemotherapy.

Patients were included in the study if the predominant clinical complication of the tumor was local-regional pelvic disease. Patients were excluded only if they had pre-existing myocardial disease precluding the use of doxorubicin. Twenty-five patients had tumor arising in the bladder, one in the ureter, two in the prostatic ducts, and one in the urachus. All patients were initially evaluated with a complete history and physical examination and metastatic surveys consisting of chest X-rays, bone scan, liver-spleen scan, and iv pyelogram. Abdominal computed axial tomography, ultrasound, and lymphangiography were often employed. Baseline cardiac scans with ejection fractions were performed. Initial blood tests included a complete wbc count, differential and platelet counts, coagulation profile, and measurement of serum glucose, creatinine, BUN, phosphate, calcium, total protein, albumin, bilirubin, electrolytes, and lactate dehydrogenase.

Treatment was initiated in all patients between October 1, 1979, and March 1, 1981 (table 1). Four patients were females and 25 were males. A cystectomy was performed in only eight patients (27%) prior to pelvic recurrence and eventual referral for therapy. The mean age of the treated population was 60.4 years (median, 63; range, 39–74). Twenty-two patients had obstructive uropathy, while six had edema of the lower extremities in addition to the uropathy. Sixteen patients (55%) had received primary radiation therapy prior to referral. The clinical presentations of the treated patients are outlined in table 2. The patients were treated by the Departments of Medicine, Urology, and Diagnostic Radiology at The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute. Pathologic specimens were reviewed by the Department of Pathology and were classified by histologic subtype.

The chemotherapy regimen consisted of three agents: 5-FU, doxorubicin, and mitomycin. Doxorubicin and mitomycin were each administered iv on 2 consecutive days. Doxorubicin (25 mg/m^2) was administered on Days 1 and 2 of each course, while mitomycin (5 mg/m^2) was administered iv on Days 3 and 4.

Bilateral hypogastric catheters were placed by the Department of Radiology on Day 1 of each regimen. The catheter placement was verified by radionuclide angiography, which enabled us to determine the proportion each catheter was responsible for infusing in the clinically determined tumor bed. This allowed us to divide the total 5-FU dose into each hypogastric catheter by the amount each was responsible for infusing in the tumor bed. Criteria for administration of each course were a granulocyte count $\geq$ 2000/mm^3, a platelet count $\geq$ 100,000/mm^3, and the absence of clinical, echocardiographic, radiographic, or electrographic changes suggesting doxorubicin-induced cardiomyopathy.

The patients initially received three courses of combined intra-arterial and systemic chemotherapy at a minimum interval of 4 weeks. Each patient then received the iv combination regimen at increasing time intervals, which were determined by marrow recovery.

Survival curves were prepared by the Kaplan-Meier method, and comparison between curves was

TABLE 1.—Pretreatment patient characteristics

Characteristics	No. of patients
Total in study	29
Sex	
Males	25
Females	4
Age (yrs)	
Median: 63	
Mean: 60.4	
Range: 39–74	
Prior radiation therapy	16 (55%)
Prior cystectomy	8 (27%)
Edema, lower extremities	6 (20%)
Obstructive uropathy	22 (76%)

TABLE 2.—Clinical presentations of 29 patients

Presentation	No. of patients (%)
Pulmonary metastasis	2 (6.8)
Unilateral hydronephrosis	15 (52)
Bilateral hydronephrosis	7 (24)
Edema, lower extremities	6 (20)
Renal failure (creatinine > 2 mg/100 ml)	5 (17)
Liver metastasis	1 (3)
Rectal invasion	5 (17)
Bone metastasis	1 (3)

TABLE 3.—Responses by histologic subtype

Histology	No. of patients	
	Total	Responding (%)
TCC*	20	12 (60)
Adenocarcinomatous transformation (TCC)	1	1
Squamous transformation (TCC)	3	0 (0)
Adenocarcinoma†	4	4
Spindle variant	1	0
Total	29	17 (58)

*TCC = transitional cell carcinoma.
†Signet ring; mucin producing.

made with the Gehan modification of the Wilcoxon two-tailed test. Response criteria were simple. Patients who achieved an objective decrease in tumor size, as determined by radiographic criteria or by examination under anesthesia, were considered to have responded. Four of the 17 patients who responded had a decrease in tumor size, as shown by computed axial tomographic scan. One patient had relief of a small bowel obstruction, which was demonstrated by an upper gastrointestinal series; two had a decrease in urinary obstruction, as demonstrated by iv pyelogram; two had a marked improvement or disappearance of an infiltrating penile mass; and one had disappearance of neoplastic vaginal deposits. Each of the remaining seven responding patients had improvement or disappearance of disease, as shown by rectal or pelvic examination and cystoscopy. Repeat cystoscopy was performed in six patients, and four had disappearance of all measurable disease.

RESULTS

Seventeen patients (58%) achieved an objective response. The response rates by histologic subtype are shown in table 3. Twelve (60%) of 20 patients with transitional cell carcinoma responded. All four of the patients presenting with one of the adenocarcinoma subtypes responded, whereas the three patients with squamous transformation of transitional cell carcinoma failed to respond. The patient with a spindle variant failed to respond, while the patient with adenocarcinomatous transformation of a transitional cell carcinoma responded.

Four patients had distant visceral metastases in addition to local pelvic disease. One of the two patients with lung metastases responded with a > 50% reduction of a pleural-based nodule. A patient with liver metastases was treated with hepatic infusion of 5-FU after maintaining stable disease during the pelvic infusion of chemotherapy. The patient with bone metastases failed to respond.

Of the six patients who had repeat cystoscopic examinations, four had no evidence of residual local tumor, and two had persistent viable malignancy. Of the four with negative cystoscopic examinations, two were only visually inspected and two had negative biopsies in the previous tumor bed. Two of the patients with negative cystoscopic examinations eventually failed with hepatic metastases and upper para-aortic and supraclavicular nodal disease, without developing recurrent pelvic disease. Of the two patients who did have residual cancer on repeat cystoscopy, one had multiple superficial tumors, while the second had residual tumor in a persistent mass that was reduced > 50% in size at 1 year following initial treatment.

A third patient developed a second primary adenocarcinoma of the lung with no evidence of recurrent bladder cancer at 16 months following initial therapy.

Toxic effects are shown in table 4. One patient died as a result of chemotherapeutic toxicity. Death was due to respiratory failure, which at post-mortem examination was attributed to a previously unsuspected mitomycin lung toxicity. A second patient developed clinical mitomycin-induced interstitial pneumonia and responded to steroid therapy. One patient died in status asthmaticus following completion of two courses of infusional therapy. This

TABLE 4.—Toxic effects of intra-arterial chemotherapy for malignant urothelial tumors

Toxic effect	No. of patients (%)
Chemotherapy-related deaths	1 (3)
Motor neuropathy	4 (14)
Localized inflammatory skin changes*	29 (100)
Leukopenic fever	17 (59)
Mitomycin lung toxicity	2 (7)
Catheter placement complication†	3 (10)
Stomatitis	11 (38)

*Erythematous eruption in the distribution of the gluteal vessels. 9 patients (11%) had bullae skin changes.
†Arterial embolus, subintimal hemorrhage.

patient had a prior history of asthma, and his death was not attributed to the chemotherapy.

Inflammatory skin changes were encountered in the distribution of the gluteal vessels. The inflammatory skin changes, which were attributed to 5-FU, increased in severity with the number of courses infused. Most of the patients had painful erythematous reactions, while nine had localized bullous changes, which eventually resulted in superficial desquamation.

The sites of cutaneous toxicity could be predicted by radionuclide angiography. The drug concentration via the catheter that demonstrated the most cutaneous uptake was decreased where possible without compromising the drug delivered to the target site. Patients received meticulous care of the skin lesions, with frequent use of bactericidal cream (silver sulfadiazine, Silvadene) during the erythematous phase of cutaneous toxicity. If bullae developed, wet-to-dry soaks with 1:40 Burow's solution were used liberally. The use of air mattresses and frequent bedding changes avoided further deterioration in the drug-induced skin changes. No permanent skin ulceration was encountered, although frequently there were areas of residual hyperpigmentation at the site of prior cutaneous toxicity.

Three patients had complications of catheter placement. All patients received further intra-arterial chemotherapy, and each recovered with conservative medical management.

The survival time of the 17 responding patients was compared to that of the 12 nonresponding patients. The median survival of the responding patients was 52 weeks, while that of nonresponding patients was 28 weeks (fig 1). The median survival of patients with hydronephrosis was 32 weeks compared to 47 weeks for those without hydronephrosis ($P = 0.05$). The most profound negative effect on survival was the combined presence of hydronephrosis and edema of the lower extremities (fig 2).

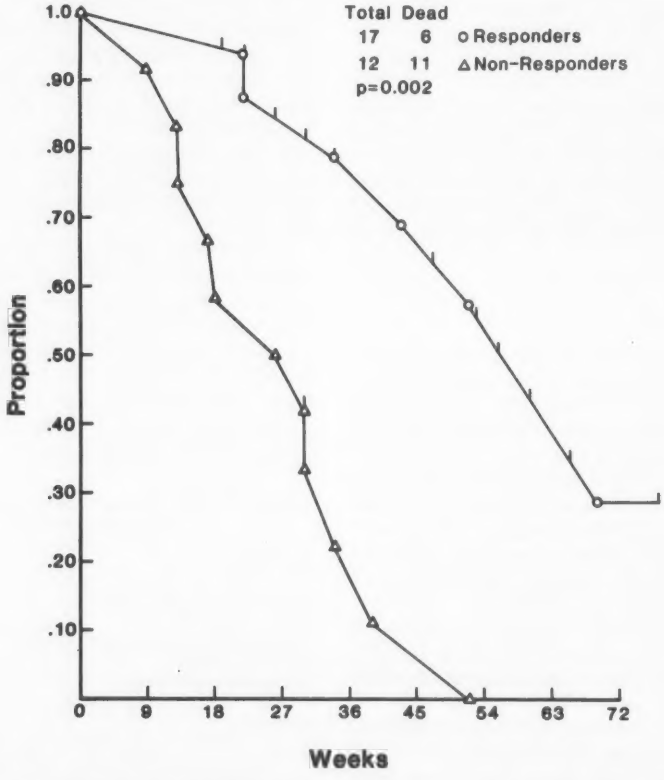

FIGURE 1.—Survival comparison of responding to nonresponding patients.

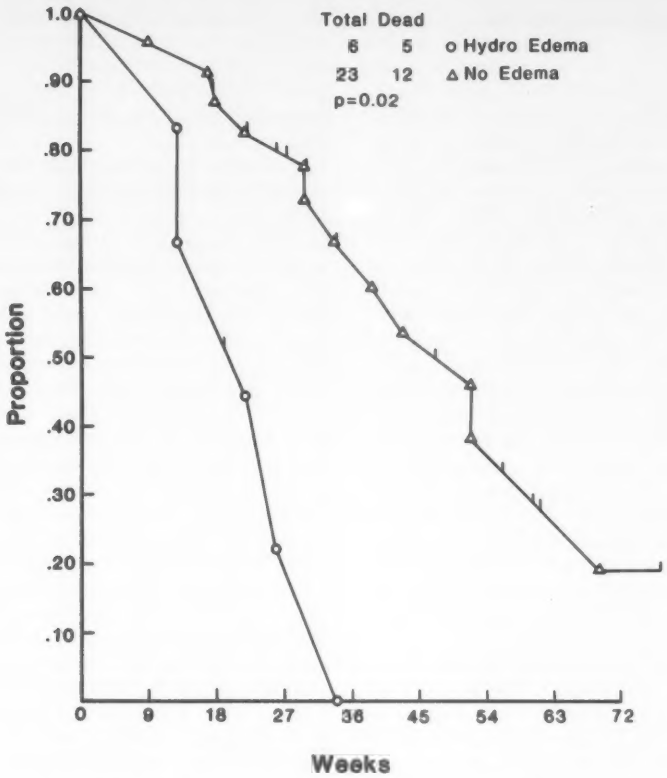

FIGURE 2.—Survival comparison of patients presenting with hydronephrosis and edema of the lower extremities (○) and the remaining population (△).

DISCUSSION

Pelvic recurrences of malignant urothelial tumors are among the most difficult and poorly tolerated clinical circumstances in genitourinary oncology. Symptoms usually result from malignant invasion of the sacral plexus, external genitalia, or urinary bladder.

Cisplatin is reported to be the single most active agent in the treatment of transitional cell carcinomas (10). Its use is often restricted because of renal insufficiency in this population with advanced age and the frequent complication of obstructive uropathy. Mitomycin, a modestly nephrotoxic agent, has previously been reported to be active in the treatment of bladder carcinoma. Early et al (11) reported a 21% response rate in 1973, and varying response rates ranging from 10% to 33% have been reported (12). 5-FU has been reported to be active as a single agent when administered iv. The most gratifying responses with 5-FU in the treatment of bladder carcinoma have been seen when it is used intra-arterially (13). Response rates with doxorubicin have varied greatly in different reports, with initial studies reporting a 38% objective response rate (14,15).

In the treatment of these select patients who required rapid local control of pelvic symptoms, we adopted a combined intra-arterial and systemic chemotherapy regimen. 5-FU was chosen as the single agent to be administered intra-arterially at a maximum tolerated dose.

The hypogastric arterial system, which is responsible for the arterial supply to the true pelvis, is readily approachable with the use of percutaneously placed arterial catheters. Nevin et al (13) have documented the effectiveness and toxicity of this approach using 5-FU in combination with irradiation. In our study, serious toxicity could be attributed to catheter placement, leukopenia, 5-FU

infusion, or the idiosyncratic lung toxicity associated with mitomycin. Catheter placement in the hands of our Department of Diagnostic Radiology has been associated with a relatively small complication rate. In 78 catheter placements, three patients had complications directly related to the arterial catheters. Two of the patients had subintimal injections into their hypogastric arteries, with partial occlusion of the involved vessel, while one patient had an embolus to the left lower extremity. All patients were treated conservatively and received further infusion without significant complications. The high rate of leukopenic fevers (59%) can be attributed to the simultaneous presence of localized inflammatory skin changes and leukopenia. Four patients had 5-FU-induced transient motor neuropathy; all of these patients recovered completely. The one chemotherapy-related death was a result of respiratory failure; the patient died of mitomycin-induced interstitial pneumonia. The 29 patients received 1000 mg/m^2 of 5-FU intra-arterially for 5 consecutive days, which is the maximum tolerated dose. The dose-limiting toxic effect of this combined iv and intra-arterial regimen is the cutaneous inflammation attributed to local 5-FU toxicity.

The effectiveness of the combined intra-arterial and iv chemotherapy is evident when the survival durations of responding versus nonresponding patients are compared (fig 1). The median survival of the responding patients was 52 weeks, whereas the nonresponding patients had a median survival of 28 weeks, a significant difference ($P = 0.002$). The extent of disease greatly influenced the effectiveness of chemotherapy and the ultimate duration of survival. The patients with hydronephrosis had a significantly shorter survival than the remaining population ($P = 0.05$). We regard edema of the lower extremities and hydronephrosis as evidence of extensive local disease. The median survival in these patients was only 20 weeks and was not favorably influenced by the chemotherapy.

The patients who benefited most from the chemotherapy had initially presented without evidence of edema of the lower extremities. These patients had only local pelvic or small para-aortic nodal disease. Five of these patients had a repeat cystoscopy because they had no remaining measurable disease. One patient underwent cystoscopy for a radiographic abnormality thought to be a residual scar. Four of these six patients were found at cystoscopy to have no viable cancer, while two have active carcinoma, as shown by biopsy. Of the four with negative cystoscopic examinations, two developed distant metastatic disease while maintaining local control and two had no evidence of recurrent disease at 9 and 13 months. Of the two patients with residual carcinoma, one had multiple superficial tumors, while the second had a > 50% reduction in the size of his pelvic tumor and is asymptomatic on continued chemotherapy at 14 months following initial therapy.

The influence of histologic subtypes on eventual chemotherapeutic response is striking despite the small number of patients. Our data, as well as those of Nevin et al (13), strongly suggest that 5-FU is an active agent in adenocarcinoma of urothelial origin. The lack of any response among the three patients with squamous transformation of a transitional cell carcinoma suggests resistance of this histologic subtype to 5-FU-based chemotherapy.

Our experience with this select population demonstrated the effectiveness of intra-arterial 5-FU with simultaneous doxorubicin and mitomycin iv for the palliation of pelvic symptoms. Use of this 5-FU-based regimen is indicated in patients with adenocarcinomas of the urachus or urothelium and patients who either cannot receive cisplatin or have failed cisplatin-based regimens (16).

The ability of this combination to achieve a long-term response with durable control of local pelvic disease suggests a future role for pelvic infusional chemotherapy in the treatment of bladder cancer. Further studies are required to determine how best to integrate hypogastric infusional therapy, surgery, radiation therapy, and systemic chemotherapy in the treatment of bladder cancer.

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Phase I Study of Indicine *N*-Oxide in Patients With Advanced Cancer[1,2]

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SUMMARY

Indicine *N*-oxide is a pyrrolizidine alkaloid isolated from *Heliotropium indicum*, one of the widely used herbs in Ayurvedic medicine. Thirty-seven patients with solid tumors received the drug: 15 men and 22 women (mean age, 53 years). All had had prior chemotherapy, and 25 had had prior radiotherapy. Eighty-four percent had a performance status of 0–3 (Cancer and Leukemia Group B criteria). The drug was given as a short infusion over 15 minutes and repeated with a median interval of 4 weeks. Doses were escalated from 1 to 9 g/m². A total of 55 courses were evaluable. Dose-limiting toxic effects were leukopenia and thrombocytopenia, and the toxicity was cumulative with repeated doses. Other toxic effects included nausea and vomiting, anemia, and hepatic dysfunction. The hematologic toxicity tended to be more pronounced in patients with hepatic dysfunction, poor marrow reserve, and heavy prior chemotherapy and radiotherapy. There were no complete or partial responses. One patient with skin melanoma and another with ovarian carcinoma had improvement lasting 2 months. The maximally tolerated dose is 9 g/m² in our population. A recommended dose for therapeutic study is 7 g/m². High-risk patients should be started at a dose of 5 g/m². The treatment may be repeated at 4-week intervals with close monitoring of wbc and platelet counts. Dose reductions may be necessary for repeated courses.

[Cancer Treat Rep 66:1509–1515, 1982]

Indicine *N*-oxide is a water-soluble pyrrolizidine alkaloid isolated from *Heliotropium indicum* (1). This is one of the most widely used herbs in Ayurvedic medicine in India and in some other countries. Pyrrolizidine alkaloids were generally shown to be hepatotoxic; the digestion of such alkaloids by grazing animals resulted in chronic liver disease. This observation led to the study of their chemical and toxicologic properties. While the alkaloids produced antimitotic and mutagenic activity, as well as chromosomal breakage indicative of possible antitumor activity (2,3), the hepatotoxic effect of the compound dampened the enthusiasm for their clinical trials (3,4). Indicine *N*-oxide produced little hepatotoxicity in rodents and was active against a variety of experimental animal tumors including P388, L1210, and P1534 leukemias, B16 melanoma, and Walker 256 carcinosarcoma (1,5). It showed a route and schedule dependency. Thus, in the P388 system, the greatest degree of activity was provided by daily ip treatments for 9 consecutive days (q1d Days 1–9).[4] This schedule was ineffective by sc or oral administration of the drug. Given orally, the *N*-oxides of pyrrolizidine alkaloids produced toxic-

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[4]Clinical brochure: indicine *N*-oxide (NSC 132319). Prepared by the National Cancer Institute, Bethesda, MD, Sept 1977.

ity comparable to that of the free base, presumably because of reduction of the drug to the free base by the intestinal flora and subsequent metabolism to reactive pyrrolic compounds, the dehydropyrrolizidines, by the liver (6–8).

Toxicologic studies in large animals revealed the following dose-related and reversible organ system toxic effects: bone marrow hypoplasia with decreased wbc and rbc precursors; gastrointestinal toxic effects including anorexia, emesis, and hemorrhagic diarrhea; renal dysfunctions with elevated BUN and creatinine and nephrosis; and hepatic disorders manifested by elevated SGOT and SGPT and sulfobromophthalein (Bromsulphalein) retention (9,10).[4] Histopathologic evaluation of liver sections was, however, reported to be negative. Doses given within a short time interval were more toxic than equivalent doses given over a longer time. In dogs given a single dose, toxic dose low (TDL), toxic dose high (TDH), and lethal dose (LD) were 3, 12, and 24 g/m^2, respectively. On a daily × 5 schedule, lower total doses were tolerated.

Indicine *N*-oxide is the first pyrrolizidine alkaloid to be studied as an antitumor agent in humans. In concert with the effort by the National Cancer Institute (Bethesda, MD), three different schedules were explored by different institutions: single dose every 3–4 weeks (11,12), weekly dose (11), and daily dose for 5 consecutive days (13–15). Earlier clinical data have been reviewed (16). The present report describes our clinical experience (12) with the compound administered as an intermittent single iv bolus in patients with advanced cancer.

PATIENTS AND METHODS

Indicine *N*-oxide was supplied by the National Cancer Institute as a lyophilized powder, 1 g in a 20-ml vial. The drug was stored under refrigeration. The initial dose of 1 g/m^2 as a single iv injection was selected on the basis of large-animal toxicologic data (9,10) corresponding to ⅓ TDL in beagle dogs. Immediately prior to use, 1 g of the drug was dissolved in 20 ml of 5% dextrose in water or normal saline and administered iv as a rapid infusion over 15 minutes.

There was spaced entry of patients to minimize the risk of toxicity for each subsequent patient. After observing the course of three patients at the initial dose level, subsequent cohorts were entered at progressively higher dose levels, guided by a modified Fibonacci search scheme. At the dose level that produced moderate but non-dose-limiting toxicity, additional patients were entered. In the absence of dose-limiting toxicity, the dose was escalated in subsequent groups of patients as well as in subsequent courses for the same patients.

Criteria for entry in the clinical trials included the following: histologic diagnosis of metastatic cancer refractory to conventional therapy; adequate hematologic function with a peripheral wbc count $\geq$ 4000/μl and a platelet count $\geq$ 100,000/μl; normal renal function (BUN $\leq$ 30 mg/dl and creatinine $\leq$ 1.5 mg/dl); adequate hepatic function (bilirubin $<$ 1.2 mg/dl and hepatic transaminases $<$ 50 milliunits/ml). In the latter phase of the study, patients with mild pre-existing hepatic and renal dysfunctions, as well as those with fluid accumulation causing a moderate third space, were included at lower doses to evaluate the influence of such dysfunctions on drug effects and vice versa. They included four patients with creatinine in the range of 1.7–2.1 mg/dl, three with mild liver dysfunction (SGOT $\leq$ 93 milliunits/ml, SGPT $\leq$ 74 milliunits/ml, and bilirubin $<$ 1.2 mg/dl), and seven with ascites and/or $\geq$ 2+ leg edema constituting third-space fluid. Baseline studies included physical examination, body height and weight, cbc and complete automated chemical profile (SMA-6 and SMA-12), urinalysis, chest X-ray, and measurement of tumor lesions. Informed consent was obtained from all patients. Therapeutic effects were evaluated using our standard criteria (17).

RESULTS

Thirty-seven patients (15 men and 22 women) with advanced cancer were included in the study. Their ages ranged from 21 to 75 years (median, 55; mean, 53). All had received prior chemotherapy, and 25 had also received radiation therapy prior to entry in the study. The spectrum of diseases treated is listed in table 1. The patients were entered in the study after reversible toxic effects (except alopecia) from prior therapy had subsided. Their performance status (Cancer and Leukemia Group B criteria) was 0 (normal performance) in four patients, 1+ (ambulatory) in seven, 2+ ($<$ 50% of waking time in bed) in ten, 3+ ($>$ 50% of waking time in bed) in ten, and 4+ (100% bedridden) in six.

Three of the 37 patients entered were lost to followup for evaluation of toxic effects. Thirty-four patients received 55 courses, 14 of whom received subsequent courses. The median interval between courses was 26 days (range, 20–48). All of the 37 patients were analyzable for immediate side effects, two of whom died in $<$ 2 weeks (on Days 10 and 12). Both patients had disease progression, and neither death was judged clinically to be drug-related. Delayed toxic effects were analyzed in 32 patients who were observed for 2 weeks to $>$ 3 months.

The major toxic effect was myelosuppression. Dose

TABLE 1.—Spectrum of diseases treated with indicine *N*-oxide

Disease	No. of patients
Breast carcinoma	7
Colon carcinoma	4
Lung carcinoma*	4
Squamous cell carcinoma, head and neck	4
Sarcoma†	4
Renal cell carcinoma	2
Adenocarcinoma, salivary glands	2
Melanoma	2
Mesothelioma	2
Adenocarcinoma, ethmoid sinus	1
Gallbladder carcinoma	1
Ovarian carcinoma	1
Esthesioneuroblastoma	1
Transitional cell carcinoma, kidney	1
Adenocarcinoma, unknown primary	1
Total	37

*Squamous cell carcinoma, 2; adenocarcinoma, 1; and oat cell carcinoma, 1.

†Chondrosarcoma, 2; osteosarcoma, 1; and Ewing's sarcoma, 1.

escalation and occurrence of hematologic toxicity are summarized in table 2. While the degree of myelosuppression was progressively more pronounced at higher doses, severe leukopenia and thrombocytopenia were noted sporadically at intermediate doses. Thus, at a dose of 5 g/m^2, 13 courses of treatment were well-tolerated with no myelosuppression; however, one patient who had tolerated a dose of 3.3 g/m^2 without myelosuppression developed severe leukopenia and thrombocytopenia after a second course, given on Day 49 at a dose of 5 g/m^2. His wbc count nadir was 550/μl and his platelet count nadir was 14,000/μl. He remained leukopenic (< 4000 cells/μl) for 5 days and thrombocytopenic (< 100,000 cells/μl) for 21 days. He became febrile and received iv antibiotic therapy and a wbc transfusion. He also developed hepatic dysfunction with maximal bilirubin of 5.9 mg/dl, SGOT of 74 milliunits/ml, and SGPT of 75 milliunits/ml, which returned to normal by Day 34 (table 3). At a dose of 7.0 g/m^2, four of 16 initial courses and two of eight second courses were accompanied by severe myelosuppression (wbc count < 2000/μl and/or platelet count < 50,000/μl). Of these six courses, five were in patients with bone marrow or hepatic dysfunctions, the latter either pre-existing or drug-related. At a dose of 9 g/m^2, two of three patients had wbc count nadirs < 1000/μl and platelet count nadirs < 30,000/μl. They became febrile and were treated with iv antibiotics. One patient developed petechiae and required a platelet transfusion. With these observations, we concluded that we had reached the maximally tolerated dose in our patient population. The time to reach the nadirs of wbc and platelet counts did not differ between initial and subsequent courses. The duration of leukopenia and thrombocytopenia tended to be longer in subsequent courses. Anemia in the absence of bleeding or hemolysis was also observed more frequently at higher doses.

Evaluation of the influence of various risk factors on hematologic toxicity is summarized in table 4. Patients with heavy prior chemotherapy and radiotherapy, prior exposure to nitrosourea, decreased bone marrow reserve, or hepatic dysfunction appear to have developed severe hematologic toxicity.

Fourteen patients who received repeated courses of the drug were studied. Seven developed leukopenia and/or thrombocytopenia (table 5). In three patients, a repeated course at the same dose was accompanied by progressive lowering of the wbc count, and in two patients an escalation of the dose from 3.3 to 5 g/m^2 was accompanied by unexpectedly severe hematologic toxicity. These data indicate cumulative effects of the drug on the severity of myelosuppression.

Hepatic toxicity was noted in seven of 55 courses; there was mild pre-existing abnormal liver function in three patients (table 3). Hepatotoxicity is characterized by transient twofold (median) increases in basal SGOT, SGPT, and bilirubin, with the peak occurring on Day 19 (median), often accompanied by severe hematologic toxicity. Hepatic dysfunction reverted to normal in four courses within 10 days; two of the patients had pre-existing hepatic dysfunction. Of the patients who received the other three courses, one was lost to followup 21 days after treatment, one died on Day 14 of followup, and one died on Day 23 of followup.

Other toxic effects were of less clinical significance. Mild to moderate nausea and vomiting occurred in approximately one third of the courses given at a dose > 3.3 g/m^2. Transient diarrhea lasting 1 or 2 days occurred after two courses. Four episodes of low-grade fever were associated with the drug administration occurring on Days 2 and 4. Of the four patients with mild renal dysfunction (serum creatinine, 1.7–2.1 mg/dl) treated at doses of 2.0–7.0 g/m^2, neither deterioration of renal function nor any augmented hematologic toxicity was noted. Similarly, the presence of third space did not influence the degree of hematologic toxicity.

None of the patients had a complete or partial response. One patient with ovarian carcinoma had an improvement (less than a partial response) for approximately 2 months with the initial dose of 3.3 g/m^2 and the second dose of 5 g/m^2. Another patient with skin melanoma showed an improvement lasting

TABLE 2.—Hematologic toxicity of indicine *N*-oxide

Dose (g/m^2)	No. of evaluable courses	Course		Fall in Hgb > 1.5 (g/dl)*	Wbc count: Median nadir, $\times 10^3$ cells/μl (range)	Wbc count: Median No. of days to nadir (range)	Wbc count: Median No. of days < 4.0 $\times 10^3$ cells/μl (range)	Platelet count: Median nadir, $\times 10^3$ cells/μl (range)	Platelet count: Median No. of days to nadir (range)	Platelet count: Median No. of days < 10^5 cells/μl (range)
1.0	3	First	3	0	4.3 (3.5–12.1)	3 (2–6)	2 (2)	121 (117–260)	5 (3–6)	
		Subsequent	0							
2.0	3	First	2	0	6.4 (5.3–7.4)	4 (3–5)		300 (102–498)	14 (3–25)	
		Subsequent	1	0	7.1	4		288	4	
3.3	8	First	5	0	4.8 (3.6–10.8)	11 (7–18)	11 (11)	256 (120–462)	14 (4–24)	
		Subsequent	3	0	8.5 (8.2–14.8)	11 (4–18)		440 (429–462)	13 (7–19)	
5.0	14	First	5	0	7.1 (5.3–10.3)	7 (1–24)		285 (85–384)	20 (1–24)	7
		Subsequent	9	1	5.0 (0.55–11.4)	11.5 (7–25)	4 (4–6)	214 (14–350)	14 (6–24)	19 (15–23)
7.0	24	First	16	6	4.2 (0.98–12.2)	13 (4–16)	8 (1–27)	136 (10.5–398)	14 (6–23)	7 (6–13)
		Subsequent	8	3	4.3 (0.98–7.6)	13.5 (6–17)	17 (14–32+)†	108.5 (3–475)	13.5 (10–14)	17 (14–32+)†
9.0	3	First	3	2	0.9	11	5	29	12	4
		Subsequent	0		(0.8–4.0)	(8–11)	(5)	(6–226)	(8–12)	(4)

*Patients with documented bleeding not included. No episode of hemolysis was recognized.
†Wbc and platelet counts did not recover during observation period of 32 days.

TABLE 3.—Hepatic dysfunction in patients treated with indicine *N*-oxide

Dose (g/m^2)	Course No.	SGOT (milliunits/ml) Pre-treatment	SGOT (milliunits/ml) Post-treatment (Day)	SGPT (milliunits/ml) Pre-treatment	SGPT (milliunits/ml) Post-treatment (Day)	Bilirubin (mg/dl) Pre-treatment	Bilirubin (mg/dl) Post-treatment (Day)	Hepatic function returned to normal on Day—	Wbc count nadir ($\times 10^3$ cells/μl)	Platelet count nadir ($\times 10^3$ cells/μl)	Comments
3.3*	1	93	140 (27)	43	185 (22)	0.5	0.7 (22)	32	5.8	250	
5.0	2	34	74 (29)	6	75 (29)	0.8	5.9 (29)	34	0.55	14	
5.0	2	11	115 (19)	31	143 (19)	0.6	1.2 (19)	†	4.9	49	Died on Day 23 of followup
7.0*	1	44	77 (24)	67	94 (26)	0.8	1.9 (17)	27	1.6	13	
7.0*	1	54	100 (14)	57	102 (14)	0.9	2.2 (10)	†	1.8	18	Lost to followup after Day 21
7.0	1	5	115 (21)	6	109 (19)	0.4	0.9 (21)	27	12.2	299	
7.0	1	45	78 (10)	23	16 (10)	0.1	0.3 (10)	†	1.6	54	Died on Day 14 of followup

*Pre-existing hepatic dysfunction.
†Hepatic function did not return to normal during observation period.

TABLE 4.—Relative risk factors for severe hematologic toxicity

	Toxicity	
	Severe*	Moderate or none
No. of courses evaluable	11	44
No. of courses with—		
Median performance status	2.0	2.5
Hepatic dysfunction†	5 (45%)	2 (4.5%)
Prior nitrosourea	3 (27%)	6 (14%)
> 5 prior chemotherapeutic agents	7 (64%)	13 (30%)
Prior radiotherapy	9 (82%)	16 (36%)
3rd space	2 (18%)	8 (19%)
Mild renal dysfunction	1 (9%)	3 (7%)
Bone involvement	1	3
Bone marrow dysfunction	2‡	0

*Wbc count < 2000/μl and/or platelet count < 50,000/μl.

†3 patients had transient exacerbation of pre-existing mild hepatic dysfunction; 4 had hepatic toxic effects after treatment (*see* table 3).

‡Documented bone marrow dysfunction; 1 patient had hypocellularity and another had metastatic tumor; both had wbc count > 4000/μl and platelet count > 100,000/μ/l prior to treatment.

TABLE 5.—Comparison of hematologic toxicity after 1st and subsequent courses of indicine *N*-oxide*

Patient No.	Performance status	Dose (g/m^2)	Given on Day—	Wbc count nadir ($\times 10^3$ cells/μl)	Platelet count nadir ($\times 10^3$ cells/μl)
6	3	3.3		4.8	120
		5.0	32	3.9	41
7	3	3.3		10.8	262
		5.0	49	0.55	14
9	4	7.0		10.0	284
		7.0	21	0.975	3
22	2	7.0		10.7	398
		7.0	27	3.1	99
23	3	7.0		7.9	160
		7.0	27	4.4	97
		7.0	31	2.9	103
24	2	7.0		2.0	24
		5.0	38	4.9	49
29	1	7.0		3.1	222
		7.0	30	5.3	330

*No hematologic toxic effects were seen in 7 patients who had repeated courses of the drug at doses of 1–7 g/m^2; the maximum dose of 7 g/m^2 was given for 3 courses.

2 months and received four courses at a dose of 3.3 g/m^2.

DISCUSSION

The present study established that the dose-limiting toxic effect of indicine *N*-oxide in an intermittent single bolus schedule is myelosuppression and that the maximally tolerated dose is 9 g/m^2. A dose of 7 g/m^2 is tolerated in patients who have not been heavily treated with chemotherapy with or without radiotherapy. Patients with pre-existing hepatic dysfunction, heavy prior chemotherapy and radiotherapy, or borderline marrow reserve have less tolerance for the drug and should be given an initial dose of 5 g/m^2.

Hepatic dysfunction coincided with the drug administration in seven courses. This may be related

to dose and number of courses (table 3), but the number is too small to suggest any definite conclusions. Patients with hepatic dysfunction, either preexisting or subsequent to treatment, were more likely to have augmented myelosuppression. These data support hepatic involvement in the metabolism and/or excretion of indicine *N*-oxide. It has been suggested that while approximately 60% of the compound, after iv administration, is excreted in the urine as an unmetabolized form, the rest of the parent drug diffuses passively into the gut where the flora reduces the drug to indicine, a hepatotoxic form, which reaches the liver (18).

It has been reported that prior exposure to a nitrosourea is a major risk factor (13). While this appears to be true in our analysis, it is thought that the prior exposure to a nitrosourea cannot be singled out as the sole risk factor. Heavy prior chemotherapy, large doses of radiotherapy, and borderline marrow reserve due to prior chemotherapy and/or tumor involvement are all associated with severe hematologic toxicity.

In the daily × 5 schedule, a daily dose of 3 g/m^2 or a total dose of 15 g/m^2/course has been recommended (13). Tolerance of a larger dose by the daily × 5 schedule as opposed to a single bolus is in accord with animal data. In a weekly × 4 schedule, a weekly dose of 6 g/m^2 or a total dose of 24 g/m^2/course has been suggested (11). While no assessment can be made of the therapeutic advantage of one schedule over another, it appears that more of the drug can be administered per course with the daily × 5 or weekly schedule than with the intermittent iv schedule. The compound has been reported to have encouraging activity in patients with refractory leukemia receiving a daily × 5 schedule (15).

Delayed recovery of marrow stem cells was evident by severe myelosuppression after a repeated dose given as late as Day 49. It was reported that a daily × 5 schedule resulted in little cumulative effect on the plasma drug concentration by Day 5 (13). The observed marrow toxicity does not necessarily represent drug retention in hematopoietic stem cells but may, rather, indicate a sublethal injury to those cells not killed outright, which delays their recovery and makes the marrow vulnerable to a second dose. Patients given repetitive prolonged treatment with the drug require close monitoring of the hematologic toxicity.

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Phase I Clinical Evaluation of 9,10-Anthracenedicarboxyaldehyde[bis(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone]dihydrochloride (Bisantrene)[1,2]

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SUMMARY

9,10-Anthracenedicarboxyaldehyde[bis(4,5-dihydro-1*H*-imidazol-2-yl)-hydrazone]dihydrochloride (bisantrene) is one of a series of anthracenedicarboxyaldehyde compounds that has significant antitumor activity similar to that of doxorubicin in experimental mouse tumor systems. A phase I clinical study was conducted using a weekly × 3 schedule. Twenty-six patients with a wide variety of advanced malignant solid tumors received a total of 39 courses of the drug at doses ranging from 40 to 180 mg/m²/week × 3. Leukopenia was the dose-limiting toxic effect. Other toxic effects included malaise, which was observed in 49% of the courses, nausea and vomiting in 36%, fever in 21%, and phlebitis in 21%. No responses were observed in this study. For phase II studies, using this schedule, a starting dose of 150 mg/m²/week × 3 repeated every 4–5 weeks is recommended. In patients who have received minimal or no therapy, the starting dose should be 180 mg/m².

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9,10-Anthracenedicarboxyaldehyde[bis(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone]dihydrochloride (bisantrene) is one of several anthracenehydrazone analogs that was developed in an effort to identify a compound with a wide spectrum of antitumor activity resembling that of the anthracycline antibiotics but devoid of cardiotoxicity. The anthracene compounds do not possess the amino sugar portion of the anthracycline molecule, which was suggested by Adamson (1) to be responsible for the cardiotoxicity of the anthracyclines. Bisantrene has demonstrated significant wide-spectrum antitumor activity against experimental mouse leukemias and solid tumors, including L1210 leukemia, P388 leukemia, Lieberman plasma cell tumor, B16 melanoma, Colon tumor 26, and Ridgway osteogenic sarcoma (2). However, bisantrene did not show any activity against the P388/Adria leukemia or the Lewis lung carcinoma test systems. Its spectrum of antitumor activity is, therefore, similar to that of doxorubicin, and it is more active than dihydroxyanthracenedione against the Ridgway osteogenic sarcoma.

Although the precise mechanism of action of bisantrene has not been defined, preliminary evidence suggests that it is a DNA-reactive agent (2–4). In L5178Y mouse lymphoma tissue culture cells, bisantrene was a potent inhibitor of both RNA and DNA synthesis. When compared on an equimolar basis, bisantrene was approximately three times more potent than doxorubicin in inhibiting the incorporation of ^{3}H-uridine into RNA and four times more potent in inhibiting the incorporation of ^{3}H-thymidine into DNA.

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The target organs for animal toxicology studies in rats, dogs, and monkeys were the bone marrow, the gastrointestinal tract, and the lymphatic systems. Toxic effects have included excessive salivation, emesis, epitaxis, lacrimation, decreased food consumption, weight loss, bloody diarrhea, rapid breathing, distended abdomen, hematuria, tremor, hind-limb weakness, ataxia, and increases in body temperatures. Myelosuppression was the major hematologic abnormality in dogs and monkeys and affected the wbc count more than the rbc count. Changes noted in serum chemistry determinations included increased alkaline phosphatase, creatinine phosphokinase, SGOT, SGPT, BUN, creatinine, total bilirubin, glucose, cholesterol, and triglycerides. There was no evidence of alopecia or cardiotoxicity in these reports (2).

Because of the potential usefulness of bisantrene, a phase I clinical study was initiated at our institution to determine the maximum tolerated dose and toxicity associated with the administration of this compound on a weekly × 3 schedule.

MATERIALS AND METHODS

All adult patients who were entered in this study had histologic proof of advanced malignancy and were not candidates for known regimens or protocol treatments of higher efficacy or priority. An estimated life expectancy of at least 12 weeks was required, and all patients had measurable disease, although this was not a prerequisite for entry in the study. All patients were required to have adequate bone marrow function (defined as a peripheral absolute granulocyte count > 2000/mm^3 and a platelet count > 100,000/mm^3, with normal bone marrow cellularity), adequate renal function (creatinine < 2.5 mg/100 dl), and adequate liver function (bilirubin < 2 mg/100 dl). In addition, patients were required to have recovered completely from toxic effects of all previous chemotherapy or radiotherapy. Informed consent, indicating the investigational nature of this study, was obtained from all patients in accordance with federal and institutional policies.

Bisantrene was supplied by the American Cyanamid Company as a sterile lyophilized powder in vials containing 10, 80, or 200 mg of the free base of the compound with no preservatives. Bisantrene is a hygroscopic orange solid that is stable at room temperature. For clinical use, it was reconstituted with sterile water for injection (USP) and was further diluted in 250 ml of 5% dextrose in water or normal saline to be administered iv over 1 hour. The diluted orange-yellow solution was given within 2 hours of preparation.

A single dose was given weekly for 3 weeks followed by a 2-week observation period. Each course was repeated every 5 weeks. The toxic dose low in the single-dose studies in dogs was 64 mg/m^2 and one third of this dose is approximately 22 mg/m^2. However, based on phase I data available from Myers et al (5) when we began the study, we chose an initial starting dose of 40 mg/m^2. During the initial phase of the study, the drug dose was escalated by 100% until any signs of biologic activity were observed. Subsequent dose escalations were then made at 25%–50% increments until dose-limiting toxic effects were observed. At least three patients were studied at each dose level and were evaluated for at least 5 weeks before additional patients were given the next dose level. Dose escalation was allowed within patients.

During the study, each patient was closely observed and all toxic effects during courses of therapy were recorded. The patient's blood pressure, pulse, respiration, and temperature were monitored before and at the end of drug administration. Cbc with differential and platelet count were performed twice weekly and SMA-12 chemistry pattern screen, serum electrolytes, prothrombin time, partial thromboplastin time, and urinalysis were repeated at least once each week and prior to each course of therapy. Appropriate radiologic examinations and electrocardiograms were performed before treatment and repeated at least every 10 weeks. Treatment was discontinued when there was evidence of tumor progression following one or more courses of treatment. Patients were considered evaluable for toxicity if they received at least one course of therapy with 2 weeks of subsequent observation. Standard criteria of response were used to evaluate the antitumor effect of the drug.

RESULTS

Twenty-six patients (21 women and five men) were entered in this phase I study; the median age was 53 years (range, 32–75). Most of the patients had breast cancer (11 patients), but other tumor types included colon cancer (four); lung cancer (three); melanoma (three); sarcoma (two); and hypernephroma, pancreatic carcinoma, and carcinoma of unknown primary (one each). All patients had received prior chemotherapy and 50% had received three or more treatment regimens. Fourteen (54%) patients had been given radiation therapy. Approximately 85% of the patients had a Zubrod performance status of 1 or 2 and 15% had a score of 3 or 4. A total of 39 treatment courses of bisantrene were administered at escalating doses ranging from 40 to 180 mg/m^2/week × 3. The number of patients treated

TABLE 1.—Dose escalation scheme of bisantrene

Dose level (mg/m²/wk × 3)	No. of patients	No. of courses		
		Initial	Subsequent	Total
40	4	4	1	5
80	6	3	4	7
120	9	5	6	11
150	9	7	3	10
180	6	6	0	6

TABLE 2.—Nonhematologic toxicity by dose of bisantrene

	Dose (mg/m²)			
	80	120	150	180
No. of evaluable courses	7	11	10	6
No. of courses with—				
Malaise	1	5	7	6
Nausea and vomiting	2	3	6	3
Fever	0	0	4	4
Phlebitis	1	2	3	2
Anorexia	2	0	3	1
Hair loss	0	2	1	0
Diarrhea	0	1	1	0

and the number of treatment courses given at each dose level of bisantrene are shown in table 1.

Although all 26 patients were evaluable for acute toxic reactions, one patient died from progressive disease on Day 9 after having received two doses of Course 1 therapy. No drug-related toxic reaction was noted in any patient receiving < 80 mg/m²/week × 3 (table 2). The most common side effect reported by the patients was general malaise accompanied sometimes by a flu-like illness. This usually occurred after the second dose of each course of therapy and became more pronounced after the third dose. Each episode lasted about 2–3 days. Nausea and vomiting, seen in approximately one third of the patients, were generally mild. Severe phlebitis at the site of infusion occurred in eight of 39 courses administered. However, 16 of the 39 courses were administered via the subclavian vein using a long-line catheter. Therefore, the true incidence of phlebitis was 35% in the courses administered via the peripheral veins. Hypotension was not observed, although it was reported by Myers et al (5). No evidence of hepatic, renal, or clinical congestive heart failure was observed in this study.

The myelosuppressive toxicity of bisantrene is shown in table 3. Leukopenia was the major dose-limiting toxic effect. At 150 mg/m², the median lowest wbc count was 1800/mm³ and the median lowest absolute granulocyte count (AGC) was 900/mm³. The median time to occurrence of the lowest recorded wbc count was 16 days, with full recovery by Day 28. Thrombocytopenia (platelet count < 100,000/mm³) was not seen. At 180 mg/m², the median wbc count was 1400/mm³ and the lowest median AGC was 400/mm³. The median time to occurrence of the lowest recorded wbc count was 18 days, with full recovery by Day 29. A platelet count of 77,000/mm³ occurred in one patient at this dose level. This patient had

TABLE 3.—Hematologic toxicity of bisantrene

Dose level (mg/m² wk × 3)	No. of evaluable courses	Lowest counts, cells × 10³/mm³								
		Wbc count			AGC			Platelet count		
		Day	Median	Range	Day	Median	Range	Day	Median	Range
40	5	18	3.5	2.0–5.1	15	2.1	0.9–4.0	8	244	88–288
80	7	21	4.2	2.3–5.6	21	1.9	1.1–3.8	18	268	119–325
120	11	18	2.3	1.3–4.1	22	1.3	0.6–2.4	8	262	180–533
150	10	16	1.8	0.8–3.1	17	0.9	0.5–0.2	14	203	125–365
180	5	18	1.4	0.5–2.9	18	0.4	0.2–1.2	20	189	77–410

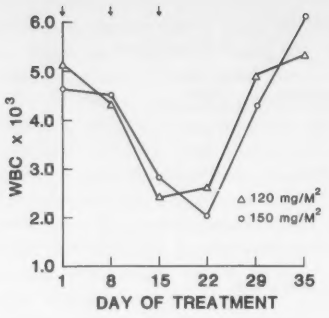

FIGURE 1.—Weekly median lowest wbc counts at 120 and 150 mg/m^2.

received extensive prior chemotherapy and radiotherapy. It was not possible to assess cumulative myelosuppression since only one patient had received more than three courses of therapy. Figure 1 shows the median lowest wbc count obtained weekly for courses at 120 and 150 mg/m^2, respectively. Maximal leukopenia occurred between Days 15 and 22 at both dose levels, with full recovery by Day 29, even though the third dose of the drug was given on Day 15.

While the major reasons for this study were to evaluate the dose and toxicity of bisantrene, observations were also made regarding antitumor activity. No objective tumor regression was observed in any of the 25 patients evaluable for response.

DISCUSSION

In this phase I clinical study, we have shown that bisantrene can be given safely to patients on a weekly × 3 schedule. The toxic effects of the drug are tolerable, predictable, transient, and reversible. The dose-limiting toxic effect was leukopenia, with very minimal effect on the platelet count. Since leukopenia is rapidly reversible (fig 1), it may be possible to administer bisantrene every 10–14 days without a rest period, as with the vinca alkaloids. The major nonmyelosuppressive toxic effect was phlebitis, occurring in 35% of the courses given via the peripheral veins. To circumvent the problem of phlebitis, we recommend the routine use of an indwelling central venous catheter, diluting the drug in 500 ml of dextrose in water or normal saline, and administering over 1 hour.

Other investigators have conducted phase I trials of bisantrene with single doses given every 3–4 weeks or daily for 5 days (5,6). The recommended dose for the single-day schedule is 260 mg/m^2 and the 5-day schedule is 80 mg/m^2/day. Although no responses were observed in our heavily pretreated patient population, it is encouraging to note that other centers have reported responses in hypernephroma, hepatoma, melanoma, adenocarcinoma of the lungs, and lymphoma. Bisantrene is, therefore, an attractive compound for further clinical studies. It has significant wide-spectrum antitumor activity in experimental animals, no cardiotoxicity in animal model systems or in this study, tolerable and reversible toxic effects, and antitumor activity in man. The results of this study suggest that for phase II studies of bisantrene, the starting dose should be 150 mg/m^2/week × 3 repeated every 4–5 weeks. In patients who have received minimal or no prior therapy, the starting dose should be 180 mg/m^2.

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Cyclophosphamide-Induced Cardiomyopathy in the Rat[1,2]

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SUMMARY

Cyclophosphamide given to ACI inbred rats produced histologic evidence of cardiomyopathy and degenerative vascular changes beginning as early as 4 days after the first of three 150-mg/kg doses (0.9 g/m^2). The interval between doses was 14 days to allow bone marrow to recover after each treatment. Twofold increases in heart weight and DNA content were observed 56 days after initial treatment; compensatory hypertrophy and a marked infiltration of the myocardium by lymphocytes were observed histologically at that time. Heart weight and DNA content were again at control levels on Day 126. Hydroxyproline content, which increased along with DNA and weight, did not decrease until after Day 196, confirming a morphologic finding of increased fibrosis after cyclophosphamide which eventually decreased in severity. These data re-emphasize the necessity for considering both the acute and chronic toxic effects, as well as recovery, when developing treatment schedules employing therapeutically effective chemotherapeutic agents.

[Cancer Treat Rep 66:1521–1527, 1982]

For the solid tumor 3924A in the ACI rat, combined therapy with cyclophosphamide and radiation is effective in producing up to 100% complete response, with ≥ 50% of these rats eventually being cured in some treatment groups (1). These schedules avoid death from acute toxicity to gut or marrow by allowing at least 11 days between drug treatments, this being the period of time necessary for recovery of bone marrow following an LD10 dose of cytotoxic drug [cyclophosphamide, 5-FU, or doxorubicin (2–4)]. To prevent recurrence of the tumor at the original site and to cure or arrest growth of metastases that were disseminated prior to local cure, a prolonged course of chemotherapy may be necessary. This may require scheduling around long-term changes in critical organs of the body, which may be different from those limiting therapy at the start of treatment.

We have initiated long-term studies (to about 1 year from treatment) to survey a number of organs (liver, gut, kidney, bone marrow, peripheral blood, spleen, heart, lung, thymus, and bladder) in the rat for damage which might limit the long-term use of cyclophosphamide in controlling metastatic growth or preventing recurrence of the primary tumor. This report summarizes our observations for the heart when ACI rats were given three doses of cyclophosphamide at 14-day intervals, each dose being the LD10 for a single dose. Parallel studies of bone marrow and peripheral blood were made to compare the time sequence of the acute effects and recovery of the hematopoietic system to the time sequence of the acute effects and recovery in the heart.

METHODS

Inbred female ACI rats (Laboratory Supply Co, Indianapolis, IN) weighing 120–140 g were given

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three ip doses of cyclophosphamide (each 150 mg/kg) at 14-day intervals. Cyclophosphamide was supplied by Mead Johnson Research Center, Evansville, IN. Groups of three rats were killed 4 and 14 days after each individual treatment and at 5-week intervals thereafter for 1 year. One hour prior to killing, each rat was given 50 microcuries of [methyl-^{3}H]thymidine (^{3}H-TdR) ip (specific activity, 3 curies/mmol). At killing, blood for wbc count, hematocrit, and hemoglobin was taken by cardiac puncture under ether anesthesia. Hearts were removed, cut transversely through the ventricles, cleared of blood, and weighed. The interventricular (septal) aspects of the ventricles were used for DNA and hydroxyproline measurements. The lateral aspect of the left ventricle was reserved for histopathologic examination.

Specimens were fixed for histology in neutral, buffered, 10% Formalin; embedded in paraffin; sectioned at 6 μ; and stained with hematoxylin and eosin. Masson's trichrome stain was used to evaluate heart muscle for fibrosis. For autoradiographs, specimens were sectioned at 4 μ, prepared with Kodak NTB-2, exposed for 2 weeks, developed with Kodak D-19, and stained through the emulsion with hematoxylin.

DNA was extracted from tissue homogenates by heating at 90°C for 20 minutes with 5% trichloroacetic acid after first eliminating RNA and small molecules by alkaline hydrolysis, precipitating with cold acid, and washing the residue twice with cold 10% trichloroacetic acid. DNA was measured by the method of Burton (5), with calf thymus DNA (Sigma Chemical Co, St. Louis, MO) as the standard.

For hydroxyproline measurements, tissue was hydrolyzed by heating at 100°C overnight in 6 N HCl. Analysis was by the procedure of Switzer and Summer (6).

RESULTS

Deaths among ACI rats given 150-mg/kg doses of cyclophosphamide on Days 0, 14, and 28 were largely confined to the 70-day period following the start of treatment (fig 1). The survival rate in figure 1 is based on the original number of rats treated, without adjustment for animals killed during the experiment, and was approximately 70% at 70 days. The 70% survival rate at 70 days is consistent with each single 150-mg/kg dose being an LD10 for the ACI rat, without cumulative toxicity, for a total dose of 450 mg/kg in 28 days. Each dose of cyclophosphamide resulted in a loss of 85%–90% of the bone marrow cells, measured as milligrams of DNA per tibia, and even greater decreases in wbc counts, particularly after repeated treatment (table 1). However, both bone marrow and wbc counts were fully restored 14 days after each treatment.

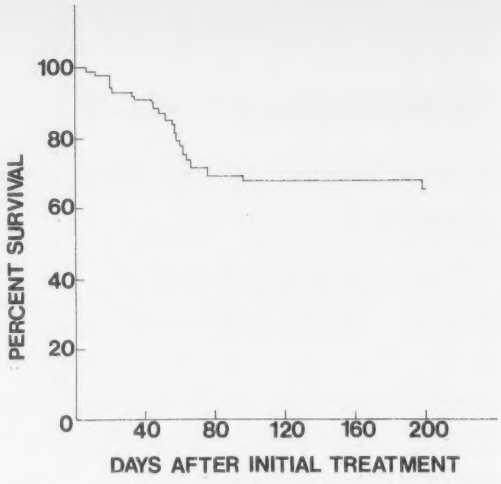

FIGURE 1.—% survival of ACI rats given 3 doses (150 mg/kg each) of cyclophosphamide on Days 0, 14, and 28.

Heart weight and DNA data for these ACI rats given three 150-mg/kg doses of cyclophosphamide are presented in figure 2. Heart weight did not change during the period of treatment but began to increase on completion of three courses of treatment; it was almost double that of untreated controls on Day 56. This increase in weight was paralleled by an increase in total heart DNA, signifying an increase in the number of nucleated cells. Histologic evaluation of these hearts revealed an influx of lymphocytes, which is the likely explanation for the DNA changes; both the lymphocytic infiltrate and the hypertrophic changes described below would contribute to the observed weight increases. Weight and DNA content of the heart returned to control levels on Day 127, and although not shown in figure 2, these were still at control values on Day 307. ^{3}H-TdR incorporation into total DNA increased shortly after the start of cyclophosphamide treatment and continued at approximately the same rate during the period of DNA accumulation, but returned to control values 91 days after treatment (table 2). Autoradiographs of hearts taken 56 days after the start of cyclophosphamide treatment revealed that nuclei of both muscle and nonmuscle cells were labeled. Labeled nuclei were rare in untreated hearts. Additional work will be needed to establish the importance of the ^{3}H-TdR labeling in cardiac muscle

TABLE 1.—Bone marrow and peripheral blood parameters after cyclophosphamide treatment of ACI rats

Days after initial dose	Total dose delivered (mg/kg)	Bone marrow DNA (mg/tibia)	Hemoglobin	Hematocrit	Wbcs	Lymphocytes	Polymorphonuclear leukocytes
0	0	0.45 ± 0.10*	13.1 ± 0.2	38.8 ± 0.2	3970 ± 750		
4	150	0.06 ± 0.002	11.6 ± 0.3	33.2 ± 0.4	530 ± 230		
14	150	0.68 ± 0.02	12.8 ± 0.4	38.3 ± 1.5	3600 ± 100		
18	300	0.06 ± 0.008	11.6 ± 0.4	35.7 ± 0.7	63 ± 13		
28	300	0.53 ± 0.04	12.1 ± 0.1	36.5 ± 0.5	4200 ± 320	1590 ± 350	2300 ± 290
32	450	0.05 ± 0.005	12.3 ± 0.4	36.0 ± 1.3	27 ± 6		
42	450	0.56 ± 0.01	12.5 ± 0.6	38.7 ± 2.2	8300 ± 2250	1810 ± 230	6160 ± 2450
56	450	0.49 ± 0.08	13.5 ± 0.9	43.0 ± 3.5	3130 ± 820	1820 ± 490	710 ± 135
91	450	0.42 ± 0.09	14.3 ± 0.2	44.7 ± 0.9	4670 ± 550		
126	450	0.20 ± 0.05	13.7 ± 0.6	42.0 ± 1.5	2770 ± 270	1400 ± 140	1130 ± 310
161	450	0.35 ± 0.07	12.6 ± 0.5	39.2 ± 1.6	2970 ± 1040		
196	450	0.14 ± 0.02	13.4 ± 0.3	39.2 ± 0.4	2530 ± 640		
231	450	0.29 ± 0.03	12.8 ± 0.4	40.0 ± 0.6	4230 ± 840		
266	450	0.28 ± 0.04	13.5 ± 0.1	41.7 ± 0.3	2100 ± 360		
307	450	0.28 ± 0.06	13.2 ± 0.2	42.0 ± 1.2	1830 ± 340		
Controls							
32	0	0.42 ± 0.04	13.3 ± 0.1	41.0 ± 0	3770 ± 200		
56	0	0.27 ± 0.08	13.6 ± 0.4	41.3 ± 1.4	3570 ± 380	2410 ± 470	780 ± 190
126	0	0.31 ± 0.03	13.7 ± 0.3	42.2 ± 0.6	3400 ± 100	1970 ± 53	1070 ± 86
196	0	0.33 ± 0.02	13.9 ± 0.0	42.0 ± 0.0	2920 ± 490		
307	0	0.50 ± 0.05	13.1 ± 0.6	42.0 ± 1.9	2300 ± 310		

*Mean ± SE for 3 animals.

cells subjected to cyclophosphamide treatment. As yet, no stimulus has been identified that will produce cardiac muscle cell proliferation in the adult heart, although interstitial cell proliferation does occur (7).

Only left ventricular tissue was available for light microscopic evaluation. Abnormal changes were noted at 4 days, the earliest time evaluated after therapy. These changes consisted of early degenerative vascular features including exudation of

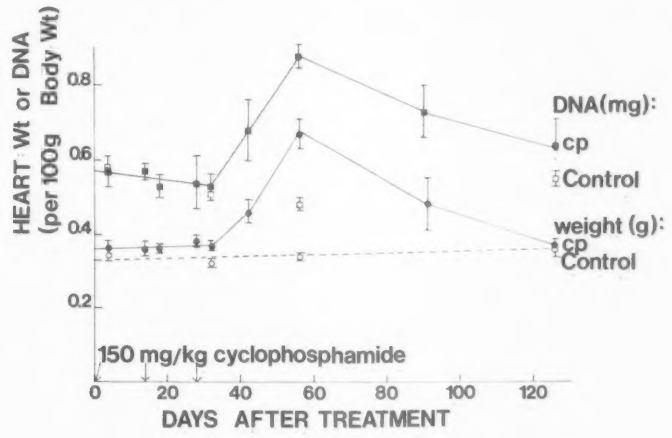

FIGURE 2.—Heart weight and total heart DNA content of ACI rats given 3 doses (150 mg/kg each) of cyclophosphamide. ■ = mg of DNA for rats given cyclophosphamide; □ = mg of DNA for control rats; ● = heart weight for rats given cyclophosphamide; ○ = heart weight for control rats. Maximum values after treatment for both weight and DNA were significantly different from control values ($P < 0.01$).

TABLE 2.—Incorporation of ^{3}H-TdR into heart DNA and hydroxyproline content of heart after cyclophosphamide treatment of ACI rats

Days after initial dose	Total dose delivered (mg/kg)	10^{-4} × dpm/mg DNA		μg of hydroxyproline/heart/100 g of body weight	
		Treated	Control	Treated	Control
0	0	—	1.85 ± 0.20*	—	186 ± 12.0
4	150	2.13 ± 0.38		180 ± 10.0	
14	150	4.27 ± 0.02		130 ± 6.7	
18	300	2.52 ± 0.22		183 ± 7.6	
28	300	8.59 ± 2.43		198 ± 14.0	
32	450	3.08 ± 0.51	1.37 ± 0.18	201 ± 4.6	171 ± 9.0
42	450	6.89 ± 1.80		307 ± 23.0	
56	450	4.82 ± 0.36	1.44 ± 0.05	410 ± 30.0†	180 ± 13.0
91	450	1.20 ± 0.29		318 ± 85.0	
126	450	1.54 ± 0.56	0.99 ± 0.11	320 ± 28.0†	227 ± 10.0
161	450	0.90 ± 0.10		405 ± 88.0	
196	450	1.26 ± 0.06	0.90 ± 0.18	414 ± 44.0†	224 ± 27.0
231	450	1.15 ± 0.07		—	
266	450	1.51 ± 0.22		321 ± 41.0	
307	450	0.88 ± 0.12	0.61 ± 0.06	241 ± 12.0	155 ± 21.0

*Mean ± SE for 3 animals.
†Different from control ($P < 0.05$).

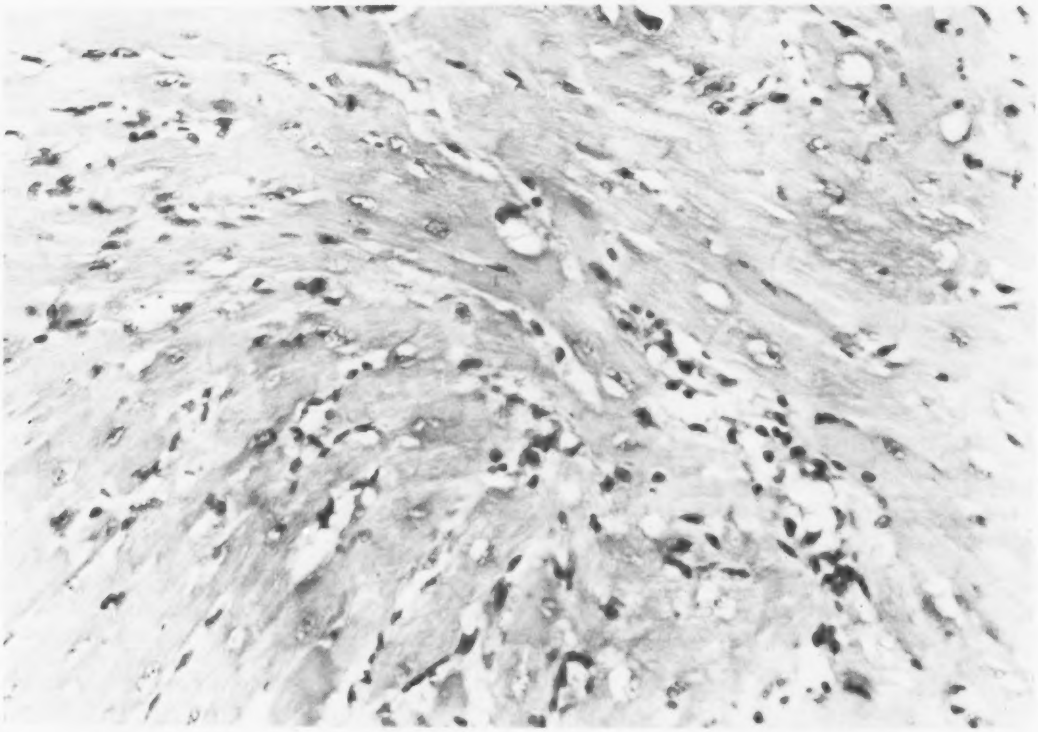

FIGURE 3.—Vacuolar degeneration of myocyte cytoplasm 56 days after start of cyclophosphamide treatment (hematoxylin and eosin stain, × 200).

lymphocytes into the perivascular wall and minimal tissue degeneration (vasculitis). Endothelial cells appeared hypertrophic, "picket-fencing" the luminal aspect of the vessel. This change was noted only focally in the left ventricular tissue and involved small arteries and arterioles. The epicardial portions of the coronary arteries were not available for evaluation. By Day 14, the endocardial surface and subendocardial connective tissues manifested vacuolar degeneration. Myocytic cytoplasmic vacuolization became noticeable at approximately Day 18. These changes increased in intensity and became more confluent. The degenerative features and a lymphocytic infiltrate increased in severity until Day 56 (fig 3). After this time the vacuolar myocytic changes appeared to lessen in severity. In actuality, markedly degenerated myocytes were replaced by early fibrosis with compensatory hypertrophy of adjacent, less-damaged myocardium. Hypertrophic changes in the adjacent myocardium were noted to a lesser degree in animals killed earlier in the experiment. Vascular changes (vascular sclerosis) noted at Day 4 progressed in severity (fig 4) and persisted throughout the extent of the experiment. Chronic tissue changes (eg, focal calcification and cartilaginous metaplasia) also increased in intensity with time for the earlier time points. However, there was less identifiable fibrotic tissue at 307 days than at either 56 or 91 days.

Hydroxyproline content of heart tissue exceeded control values on Day 42 after initiation of treatment (table 2). Twofold increases over control values were observed on Day 56 and generally persisted to Day 196, but diminished thereafter to approximately control values on Day 307. Essentially all of the hydroxyproline in animal tissues is found in collagen, the matrix substance of fibrosis in tissue (8). Thus, chemical analysis confirmed the morphologic finding of increased fibrosis after cyclophosphamide, followed by a recovery phase.

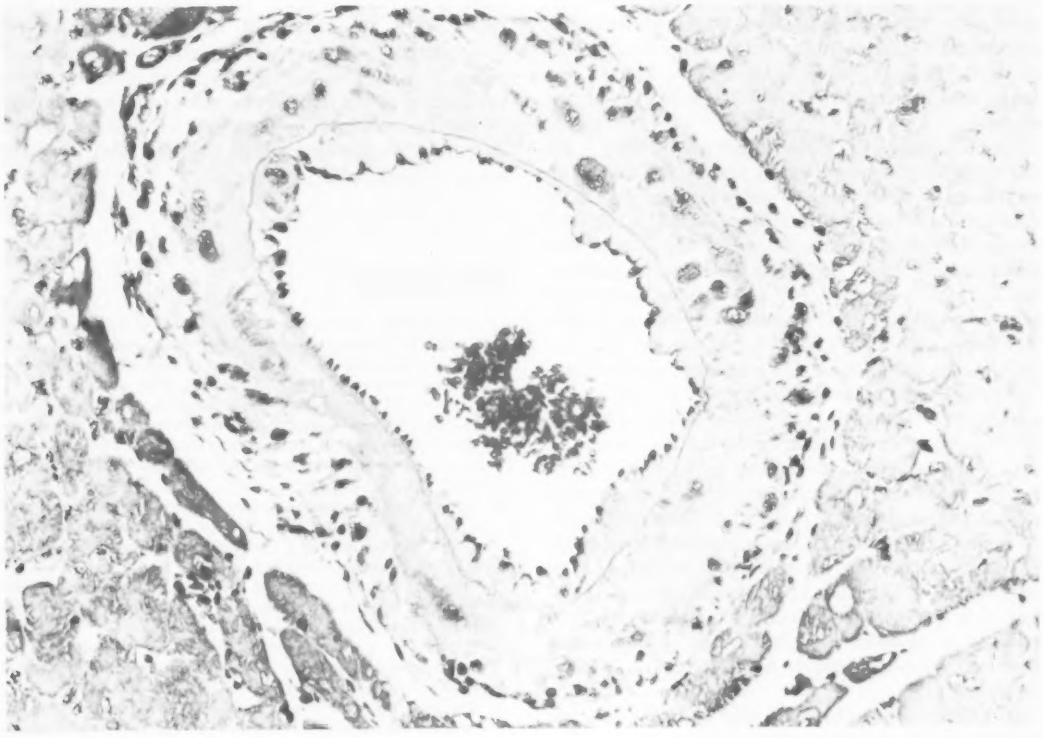

FIGURE 4.—Arterial degenerative change 126 days after start of cyclophosphamide treatment (hematoxylin and eosin stain, × 40).

DISCUSSION

The effectiveness of cyclophosphamide for therapy in man and experimental animals having a variety of neoplasms is well-established (9). The maximum dose tolerated by man is about 27–41 mg/kg (or 1.0–1.5 g/m^2), which can be repeated at 17–24-day intervals without producing cumulative hematologic toxic effects (10). With very high-dose cyclophosphamide of 40–50 mg/kg/day or more for up to 4 days, as used for intensive remission induction and bone marrow ablation prior to marrow transplantation, fatal cardiotoxic reactions have been observed (11–14). These high doses in man correspond on a surface area basis (15) to 6.7 g/m^2, for example, for the dose of 180 mg/kg in 4 days as used by Gottdiener et al (13). In contrast, the morphologic and compositional changes reported here for the ACI rat are occurring at a total dose of 2.7 g/m^2 delivered in three equal doses at 14-day intervals. This difference in total dose suggests either that the rat is more sensitive to cyclophosphamide than is man with respect to cardiac damage or that in man damage may be occurring that has so far escaped detection except in those cases where death has ensued. Some cardiac damage was observed in the rat after only one dose; however, none of the deaths that occurred could be attributed to cardiac failure.

The pathogenesis of cardiotoxicity from very high-dose cyclophosphamide therapy probably results from endothelial toxicity with transudation of the drug or its products into surrounding tissue (13,16). The morphologic changes noted here for the rat at doses corresponding on a surface area basis to conventionally used clinical doses are similar in several respects to those noted by others in human hearts after very high doses (12). The prominent vascular damage, the interstitial inflammation, and the myocytic degeneration in these animals all support the hypothesis of Buja et al (16) and of Gottdiener et al (13) regarding endothelial injury as the source of tissue damage. However, in our experiment, there was no evidence of focal hemorrhage, neutrophil infiltrate, and actual myofibrillar necrosis, which are present in acute human cyclophosphamide cardiotoxicity (17). The pathogenesis of the marked lymphocytic infiltration observed in the rat is unknown. Other drugs, especially catecholamines, are reported to produce changes which closely mimic those for cyclophosphamide cardiotoxicity in the human heart (17).

Mills and Roberts (14) have contrasted the clinical manifestations of anthracycline and cyclophosphamide cardiotoxic effects, both being dose-related but differing in that doxorubicin toxicity appears to be the cumulative result of myocardial injury, whereas the onset of cyclophosphamide cardiomyopathy is acute. Our data with the rat after relatively low total doses suggest that cumulative damage may occur with cyclophosphamide as well. A recent report (18) suggests that decreasing peak plasma levels of doxorubicin by continuous infusion over 1, 2, or 4 days reduces cardiac toxic effects without compromising antitumor activity. A similar modification in drug administration should be evaluated experimentally with cyclophosphamide, but a fatality has been reported in the clinic when a total dose of 120 mg/kg, the lowest cardiotoxic dose so far reported in man, was infused over a 24-hour period (12). Recent reports (19,20) demonstrate that cyclophosphamide toxicity that does not cause immediate death may be compensated, ending in more-or-less severe chronic cardiomyopathy, depending on the degree of cardiac damage. Echocardiographic changes, which were observed in 21 of 40 pediatric patients after high-dose cyclophosphamide treatment (20), occurred approximately 1 week after treatment and persisted for days to weeks; pericardial effusion seen in 15 of these patients was successfully treated in 13, demonstrating the reversibility of these changes with careful management. Our data in the rat also demonstrate the reversibility of certain of the compositional changes in the heart after long periods of time, with the decrease in hydroxyproline content after Day 196 being of particular interest.

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Experimental Ultrastructural and X-ray Microanalysis Study of Cisplatin in the Rat: Intracellular Localization of Platinum[1,2]

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Through the use of microanalytic techniques, it has been possible to demonstrate the presence of numerous elements in thin sections of tissue (1–3).

Cisplatin has been shown to be an effective therapeutic agent in the treatment of a certain number of types of cancers (4–7), but its use has been limited by its long-term toxicity in a number of organs including kidney, bone marrow, and liver (8–10).

Using methods of microanalysis, we have undertaken to establish the role of this element in the toxic effects manifested in the cells of different organs, ie, kidney, bone marrow (as a model of organ toxicity), and liver (as a test of platinum phagocytosis).

MATERIALS AND METHODS

Experimental Protocols

Fifteen 6-week-old male rats (Fischer 388) were treated with a weekly iv dose of 2.5 mg/kg of cisplatin (Cis-Platyl, Laboratoire Roger Bellon). The experiment was continued for 6 weeks. Controls consisted of 15 rats with the same characteristics that received weekly doses of physiologic saline solution. A similar study was conducted at a dose level of 3 mg/kg administered iv weekly.

Sample Collection

Samples of kidney, liver, and bone marrow were collected, in that order, from anesthetized rats (two treated, two controls) each week on the third day after each iv drug administration. The samples were fixed in a 1.5% glutaraldehyde solution in a pH 7.3 cacodylate buffer; some were also postfixed in a 1% solution of osmic acid in the same buffer. The samples were then washed in the cacodylate buffer, dehydrated, and mounted in Epon. Ultrathin sections of approximately 100 nm were cut using an LKB Ultro-tome III and mounted on aluminum or copper grids. None of the sections were stained.

Instrumental Methods

A Philips EM-300 electron microscope was used to study the ultrastructural morphology of the sections. A Cameca MBX electron microprobe was used to reveal the presence of the individual elements in the structure within the thin sections. The latter instrument is equipped with two wavelength-dispersive spectrometers. One is mounted inclined and is fitted with the following crystals: thallium acid phthalate (TAP), lead octadecanoate (ODPb), pentaerythritol (PET), and lithium fluoride (LiF). The vertically mounted spectrometer has PET and LiF crystals. The transmission electron microscope accessory on the electron microprobe magnifies on the order of 50,000 ×.

An ASR 33 Teletype unit is used to record X-ray signals automatically for preset counting times.

The experimental conditions used in these analyses were the following: accelerating voltage = 30 KV, beam current = 50 nA, and electron probe diameter = 500 nm.

The L_α X-rays of platinum were measured using the LiF crystal in the spectrometer, while the K_α X-rays of sulfur and iron were measured by means of the PET crystal. The X-ray counting for platinum was performed at its X-ray wavelength and background counting was accomplished at angles $\Theta + \Delta\Theta$ (0.33°). Each count was performed for 100 seconds and was repeated three times.

The statistical error in the measurement of the characteristic X-ray signal is given by $P_c = P - B$, were P = peak count signal and B = background count, and is estimated as $\sigma = \sqrt{P + B}$. The confidence interval is estimated from $P_c \pm 2\sqrt{P + B}$.

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The Student-Fisher test (t tables) (11) was used in establishing the significance of differences between the elemental signal and the background signal, based on the value of t, where

$$t = \frac{m_P + m_B}{\sqrt{\frac{s^2}{n_P} + \frac{s^2}{n_B}}}$$

and m_P = mean total count under the peak for each lysosome examined
m_B = mean background count
s^2 = standard deviation of the count (the square of the variance)
n_P = total number of countings made (three times the number of lysosomes examined)
n_B = n_p = number of background counts

and

$$s^2 = \frac{(x - m_P)^2 + (x - m_B)^2}{(n_P + n_B - 2)}$$

where x = total number of counts obtained in the preset time interval (100 seconds), m_P = average of three 100-second counts, ie, $x/3 = m_P$, and DF (degrees of freedom) = $(n_P + n_B - 2)$.

If $|t|$ is less than the value given in the table of t values for the DF at the 95% confidence level, the difference is not significant. If $|t|$ is greater than the tabulated value, the difference is significant, and the probability listed in the table for a given value of $|t|$ indicates the degree of significance.

RESULTS

In all cases, the treated rats were found to lose weight progressively with successive doses of cisplatin. Inasmuch as the results obtained at both dose levels were essentially identical in all important regards, we report only those for the lower weekly dose.

Kidney

Electron microscopy: tubular lesions.—The major changes were in the proximal convoluted tubules. They were characterized by cellular necrosis (fig 1), which engendered progressive disappearance of the contents of the cell, by the appearance of lipid inclusions in the cytoplasm, and by the presence of accumulations of dense granules in the lysosomes. These alterations all developed progressively with time.

Dense granules in the lysosomes.—Small dense granules were found in all of the samples containing lysosomes and phagolysosomes. Some of the lysosomes contained clusters of large dense granules. With the sample taken the third week (total cisplatin = 10 mg/kg), these lysosomes seemed to open directly into vacuoles in the cytoplasm. With time, the clusters of granules in the lysosomes and the number of the lysosomes increased.

Electron microscopy: glomerular lesions.—After 1 month (total cisplatin = 12.5 mg/kg), several lesions appeared in the glomerular cellular epithelium that were characterized by the presence of microvilli and a dense material (believed to consist of protein in the intercytoplasmic vacuoles) (12).

Microanalysis.—Ten series of counts were made on each lysosome containing a cluster of very dense granules (fig 3). These showed a statistically significant difference between peak count and measured background levels. The results in table 1 thus show the presence of platinum in the lysosomes of the renal tubular cells only. Microanalysis (wavelength-dispersive spectrometry) also showed the presence of the elements sulfur and iron in the same intralysosomal structures (fig 2). The wavelengths of the respective peaks for each element are such that they are easily resolved.

Bone Marrow

Electron microscopy.—Comparison of samples from treated animals with those from controls showed a break in the continuity of the adipocytes and an apparent diminution of the erythrocyte precursors.

Microanalysis.—Demonstration of the presence of iron and sulfur was attempted on reticulocytes in both the treated animals and controls. No distinction could be made between samples.

Liver

In the sections of liver studied, neither electron microscopy nor electron probe microanalysis revealed any perceptible change when samples from treated rats were compared with samples from controls.

TABLE 1.—Statistical result of counting: X-ray microanalysis*

Cisplatin dose (mg/kg)	P_c	s^2	t
20	70.97 ± 41.39	2208.44	4.09
25	84.7 ± 46.34	1905.5	4.35

*P_c = statistical error in measurement of characteristic X-ray signal, s^2 = standard deviation of count, and t = Student's t test variable. t-Table probability (3.92; DF = 18) of α = 0.001 for each dose.

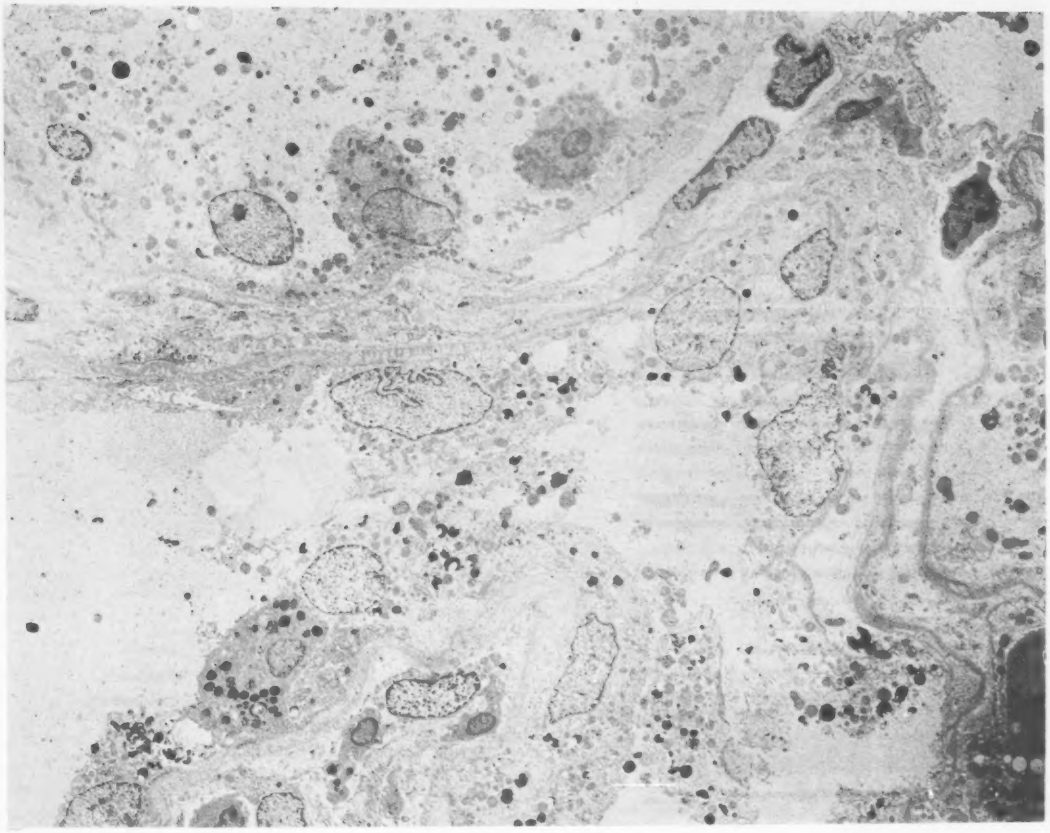

FIGURE 1.—Overview of tubular lesions in kidney of rat given 25 mg/kg of total cisplatin. Marked tubular necrosis characterized by a loss of cytoplasm. Remaining tissue is fragmented. × 3500.

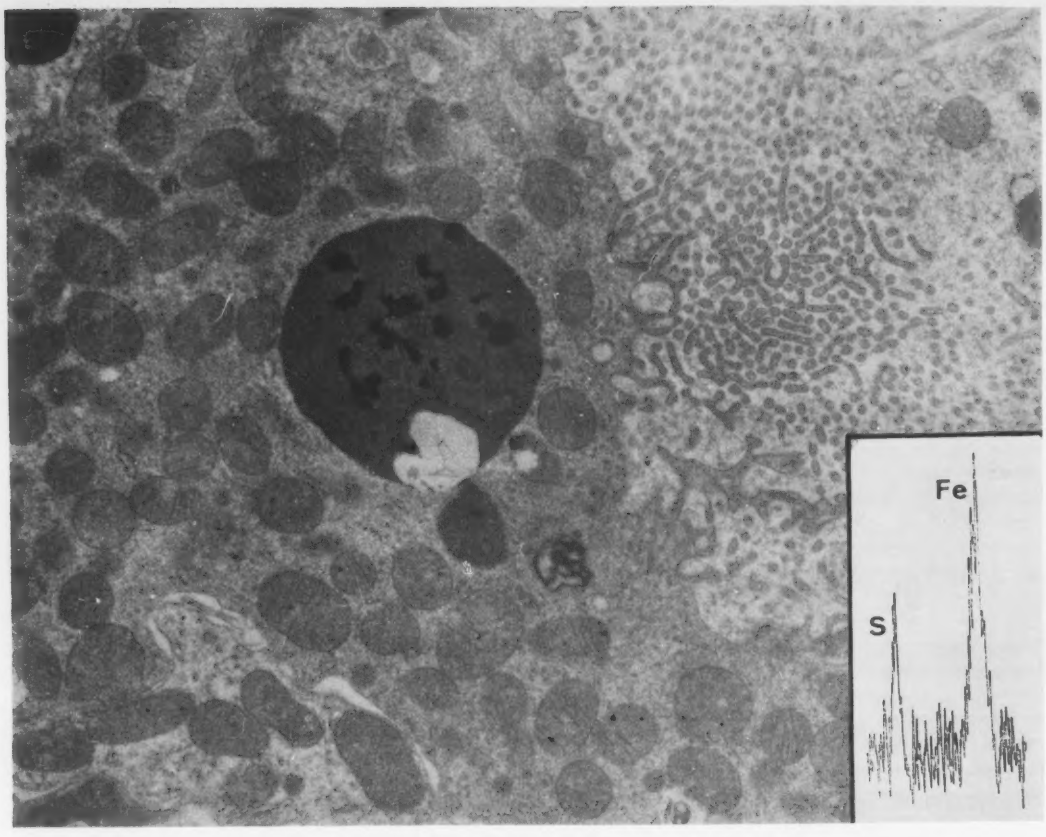

FIGURE 2.—Proximal convoluted tubule in kidney of rat given 25 mg/kg of total cisplatin. Intracytoplasmic lysosome containing dense granulations. Microanalysis showed sulfur and iron. Counts demonstrated statistically significant presence of platinum. Upper right = brush border. × 12,500.

DISCUSSION

The experimental conditions used in this study set it apart from earlier animal studies in the following respects: (*a*) use of iv injections of cisplatin in the rats (simulating the mode of administration used with man) and (*b*) use of electron microscopy and microanalysis to study chronic toxic effects.

The results obtained are similar to those obtained in other studies of metal intoxication. The metals chromium, uranium, aluminum, and gold also concentrate in the lysosomes of kidney tubules (1–3). The metallic elements may be present alone (eg, chromium) (3) or may be associated with certain other elements such as phosphorus (eg, uranium and aluminum) (1,2). Further, uranium and aluminum precipitated as a phosphate in the presence of phosphoric acid and a lysosomal enzyme. In this experiment, the platinum is accompanied by sulfur, the same element that is associated with precipitates of gold (9) and mercury, as shown in previous microanalytic studies (13).

The nephrotoxic reactions to platinum are comparable to the effects of chronic chromium intoxication. Based on this comparison, this element could be re-released from the lysosomes as they spill their contents into the cytoplasm, which becomes necrotic and is carried off in the lumen (of the tubular cells) (3).

As the experiment progressed, the platinum was present in the lysosomes and may have been released in the cytoplasm.

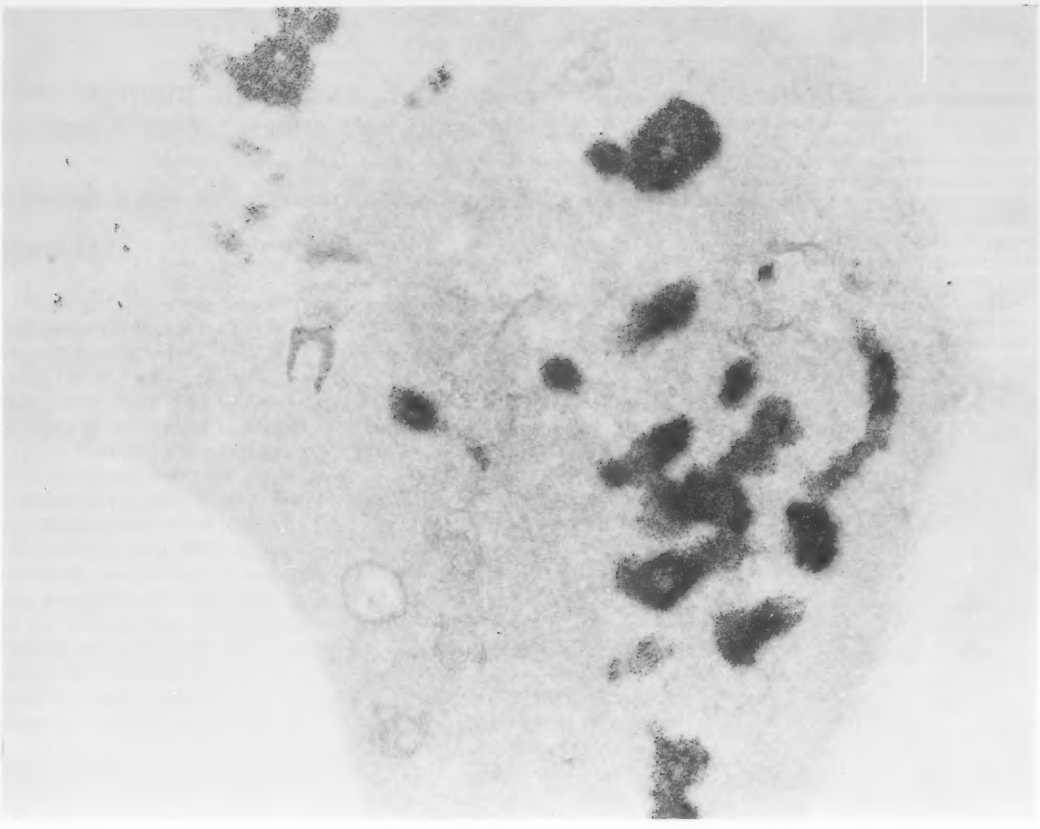

FIGURE 3.—Higher magnification (× 65,000) of fig 3, showing dense granules in lysosomal structure.

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Development and Characterization of a Cyclophosphamide-Resistant Mouse Plasmacytoma Cell Line[1,2]

Carmelita G. Frondoza,* Sudhir M. Trivedi, and Richard L. Humphrey[3,4]

SUMMARY

Murine LPC-1 plasmacytoma is sensitive to treatment with cyclophosphamide (CY), and animals bearing this tumor in advanced stages can be cured with doses as small as 60 mg/kg. With repeated transfer of LPC-1 cells followed by subcurative CY therapy, we have developed from this CY-sensitive parent line (CY-S) a stable CY-resistant subline (CY-R) that is unaffected by doses as large as 250 mg/kg. Stability of this induced CY resistance in the absence of CY treatment has been demonstrated for > 14 transfer generations. The CY-R subline has been compared to the CY-S parent line and shown to be similar with respect to morphology, growth kinetics, survival time, synthesis of IgG 2aκ M component, and DNA content and distribution. The CY-S parent line responds in vivo to CY analogs (ifosfamide and trofosfamide), as judged by decreased tumor mass and increased survival; the CY-R subline is resistant to the CY analogs. The response to carmustine (BCNU) was tested, and resistance to CY did not predict for response to this alkylating agent since the effect on both CY-S and CY-R lines was equal. The significance of the development of this CY-resistant subline and the implications for future research into the mechanisms of such resistance are discussed.

[Cancer Treat Rep 66:1535–1544, 1982]

Cyclophosphamide (CY) is a potent drug used in the treatment of plasma cell tumors (multiple myeloma) (1,2). However, patients undergoing prolonged CY chemotherapy often relapse, and subsequent treatment with this alkylating agent is no longer effective. This is a major problem in clinical oncology and severely limits successful palliation over extended periods of time. Such a pattern of acquired drug resistance has been attributed to the emergence of tumor cells that differ in their ability to evade or overcome the lethal effects of the drug. Alterations in cellular and molecular properties which may account for the development of resistance to alkylating agents have not been identified, although some biochemical differences between drug-sensitive and -resistant tumor lines such as small changes in thiol content and differences in cell permeability (ie, drug transport) have been observed (3–6). There is, therefore, a need for animal models that will allow additional studies of drug resistance at the cellular and molecular level. Knowledge of the biochemical mechanisms involved might serve as the basis for designing chemotherapeutic regimens that would inhibit transformation of drug-sensitive to -resistant tumors, avoid selection and amplification of drug-resistant cells, or restore resistant tumors to their original drug-sensitive state. Animal models could also provide tools for correlating and predicting by in vitro studies the in vivo response to alkylating agents. To date, only a few resistant sub-

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lines derived from animal tumors sensitive to alkylating agents have been developed, and no existing mouse plasmacytoma resistant to CY has been described (4–8). We have developed a murine CY-resistant (CY-R) subline from the CY-sensitive (CY-S) parent LPC-1 plasmacytoma, thus providing an animal model (particularly for multiple myeloma) that will allow analysis of changes in tumor cell properties following acquisition of drug resistance.

MATERIALS AND METHODS

Animals.—Female BALB/c mice weighing 20–25 g were obtained from Microbiological Associates, Bethesda, MD. They were given standard Charles River chow (RMH 1000) and water ad libitum.

Tumors.—LPC-1 CY-S plasmacytoma originally isolated by Dr. Michael Potter (National Institutes of Health, Bethesda, MD) was obtained from Litton Bionetics, (Rockville, MD). This tumor line was chosen as the myeloma model because of its consistent growth pattern in BALB/c mice and its production of a tumor-specific biomarker, IgG 2aκ M component. The secreted M component can easily be detected and quantitated by conventional electrophoretic methods and by more recent in vivo radiolabeling techniques (9). Tumor tissue was excised and cut into small pieces in RPMI-1640 tissue culture medium with 10% fetal calf serum (Microbiological Associates). The tissue fragments were further minced with a scalpel blade and passed through sterile gauze pads. Single-cell suspensions contained 0.2% trypan blue dye (0.2% dye in 0.9% NaCl solution). Cells were counted in a hemacytometer, and the volumes adjusted to 10^6 trypan blue-excluding cells/ml of medium. Cell morphology was examined with Jenner-Giemsa stain. The cells were maintained by serial sc or ip transfer of 10^6 cells every 3 weeks in 9-week-old female BALB/c mice.

Induction of CY resistance.—To induce a CY-R subline, mice were injected sc with 10^6 CY-S cells. Fourteen days later, mice bearing established tumors (~ 1 g) were given a subcurative dose of CY (30 mg/kg) ip. The tumors were allowed to regrow, and tumor cells were transferred 2 weeks later into another group of mice. Subsequent transfer generations were treated ip with gradually increasing CY concentrations (from 30 to 250 mg/kg). Development of resistance was followed by testing each transfer generation of cells in a separate group of ten mice with a curative ip dose of CY (125 mg/kg) on Day 14 after sc transfer of 10^6 cells.

Chemical reagents.—CY was obtained from Mead Johnson & Co (Evansville, IN). Trofosfamide, ifosfamide, and carmustine (BCNU) were donated by the Drug Synthesis and Chemistry Branch of the National Cancer Institute.

Isotopes.—6-^{14}C-arginine, 25.9 millicuries/mmol in 0.1 N HCl, obtained from New England Nuclear Corp (Boston, MA), was neutralized with 0.1 N NaOH and then diluted with an appropriate volume of 0.9% NaCl solution. Labeled amino acid (40 microcuries/mouse) was administered ip. ^{14}C-leucine, 339 millicuries/mmol in 2% ethanol, was also obtained from New England Nuclear Corp.

Collection of blood samples.—Blood was collected from the retro-orbital plexus using sterilized heparinized micropipets 24 hours following isotope administration. The micropipets were centrifuged, and the plasma obtained was frozen at –20°C until used.

Electrophoresis and radioautography.—Plasma (1 μl) was electrophoresed on Pol-E film (1% agarose/5% sucrose in 0.075 M barbital buffer, pH 8.6; Pfizer Diagnostics Division, Clifton, NJ) at 100 V for 50 minutes. The films then were soaked in 5% trichloroacetic acid containing 0.2% Ponceau S solution for 20 minutes. Excess stain was removed by washing with 5% acetic acid. The films were dried at 72°C and radioautographed using Kodak SB54 negatives for up to 4 weeks. The negatives were developed with a Model M6A-N Kodak RP X-OMAT processor. The electrophoretograms and radiograms were scanned at 525 nm with a Model 415 Clifford densitometer. The relative distribution of protein components (ie, albumin, γ-globulins, and M component) and radioactivity were determined based on the densitometric tracings. The M component region for each electrophoretic run was defined by unlabeled purified LPC-1 M component, placed in top and bottom wells of each membrane, and used as guide strips. This permitted the arbitrary assignment of radioactivity (from the radioautograms) and protein concentration (from the Ponceau S-stained membranes) to the M component, even when the M component concentration or radioactivity was too low to reveal a distinct homogeneous band.

In vitro Ig synthesis and secretion.—Viable LPC-1 cells were separated from other cells by one-step Ficoll-Hypaque gradient centrifugation (10). Tumor cell suspension (10^6 viable cells/ml) was prepared aseptically in leucine-free Eagle's minimum essential medium containing 25 mM Hepes buffer and 5000 units of penicillin-streptomycin (Microbiological Associates). Ten milliliters of the cell preparation in suspension tissue culture flasks (Bellco, NJ) was incubated with 20 microcuries of ^{14}C-leucine at 37°C. One-milliliter aliquots were centrifuged at 1000 rpm for 10 minutes, and the supernatant was obtained for electrophoresis and radioautography as

well as for analysis of total and protein radioactivity. The cell pellet was resuspended in 1 ml of medium, and aliquots were cytocentrifuged for morphologic studies or lysed by repeated freeze-thawing to determine intracellular protein radioactivity.

DNA distribution analysis by fluorescence-activated cell sorting (FACS II).—Cells were stained with propidium iodide solution containing RNase and NP40 detergent according to the procedure of Vindelov (11). The nuclear preparation was then filtered through a 30-μ Millipore filter (13-mm diameter) and was analyzed in a FACS II (Becton and Dickinson, Los Angeles, CA). Details of instrumentation have been previously described (12). The fluorescent signals were displayed as a frequency distribution histogram on a 250-channel pulse-height analyzer. For standardization, chicken rbcs (CRBC) prepared by the method of Herzenberg and Herzenberg (12) or purified normal mouse spleen lymphocytes were added to the tumor cells. The coefficient of variation ranged from 3% to 5%.

RESULTS

Development of Mouse LPC-1 CY-R Subline

The CY-R subline of the LPC-1 plasmacytoma was developed by exposure of several tumor transfer generations to palliative (subcurative) doses of CY (30 mg/kg). In this mouse model, any host variability effects by previous CY exposure were eliminated by rescuing the treated and recovered tumor cells and transferring a standard tumor cell dose (1×10^6 cells) into a fresh host. Resistance to CY was monitored during the development of this CY-R line by challenging a separate group of mice bearing sc tumor of that generation with a curative dose of drug (125 mg/kg). A prolonged period of drug exposure with as many as nine tumor passage generations was required for resistance to be detectable, and up to 18 generations were required before CY resistance was complete (fig 1). After the ninth tumor cell transfer, development of drug resistance

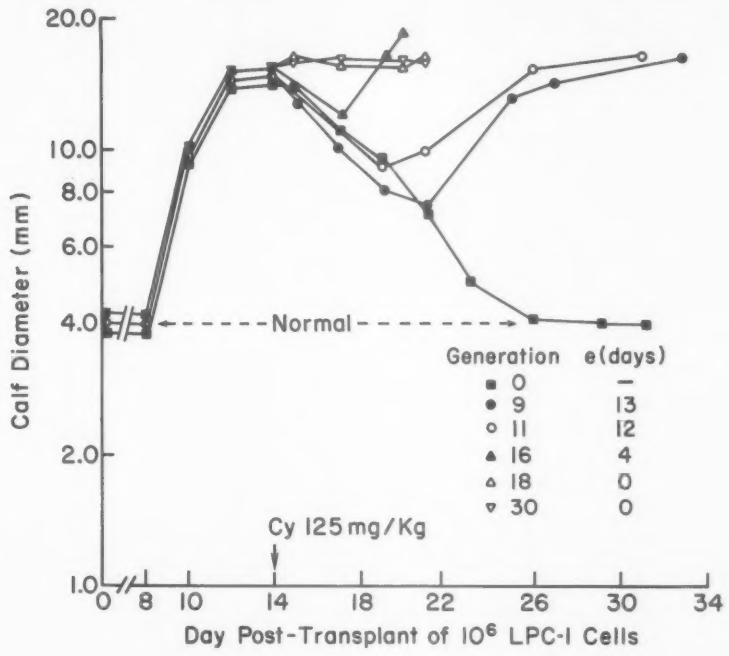

FIGURE 1.—Assay for CY resistance. Response to CY as a function of generation time. A group of 10 mice received a "curative" dose of CY ip (125 mg/kg) 14 days after sc cell transfer of 10^6 cells. Calf diameters (mm) were the mean of 2 caliper measurements taken at right angles to each other. 5 representative generations of tumor growth are shown. "e" (days) = time required for the tumor to resume pretreatment size.

TABLE 1.—Development of CY resistance

Generation No.	Median survival time (days) of mice	
	Untreated	Treated*
0†	25	> 60‡
9	25	34
18	25	25
24	25	25
35	25	25

*125 mg/kg of CY.
†Parent, CY-S.
‡"Cured."

was indicated by a transient regression in tumor volume after challenge with a single curative dose of CY, followed by regrowth (fig 1). In contrast, the parent tumor, CY-S (generation 0), completely disappeared and did not recur. The time interval (e) required for the tumor to resume its pretreatment size was shorter with each transfer generation (fig 1). By passage 18, there was no significant decrease in tumor size following a drug dose which was curative for the parent, drug-sensitive tumor of similar size. The progressive development of drug resistance was further demonstrated by the shorter survival times (25 days) seen in the later generations (table 1, generation 18 and beyond) in contrast to the earlier generations (table 1, generation 9) which displayed a prolongation of survival (34 days) but not a "cure."

Earlier generations in the development of CY resistance showed a dose-dependent response to CY (fig 2). When the lowest curative dose of CY (62.5 mg/kg) was administered, the least tumor regression and earliest recurrence were observed (fig 2). Subsequent CY-R passages (ie, by generation 18) no longer responded to these drug concentrations. Once CY resistance status was achieved, the tumor remained drug-resistant despite prolonged (> 14 generations) passage in the absence of CY exposure.

Characterization of CY-R Tumor Cells

Similar to their CY-S parent, CY-R cells induced tumors in their BALB/c syngeneic hosts with the same growth kinetics depending on the cell doses (fig 3). Both tumor lines (at cell doses of 1×10^6)

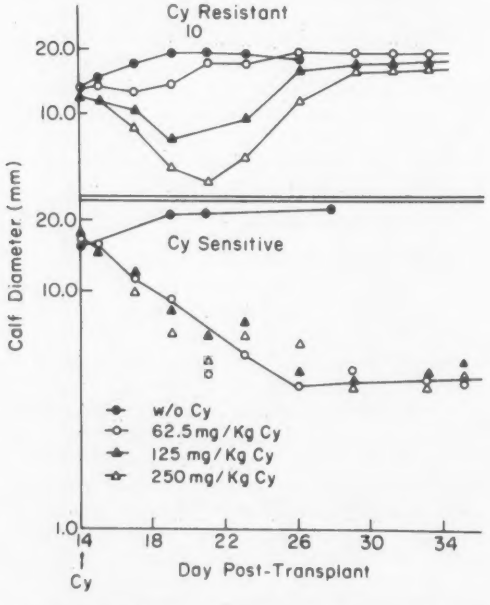

FIGURE 2.—LPC tumor growth with CY. Tumor regression (calf diameter) following curative CY treatment comparing CY-S parent line to CY-R_{10} line (generation 10).

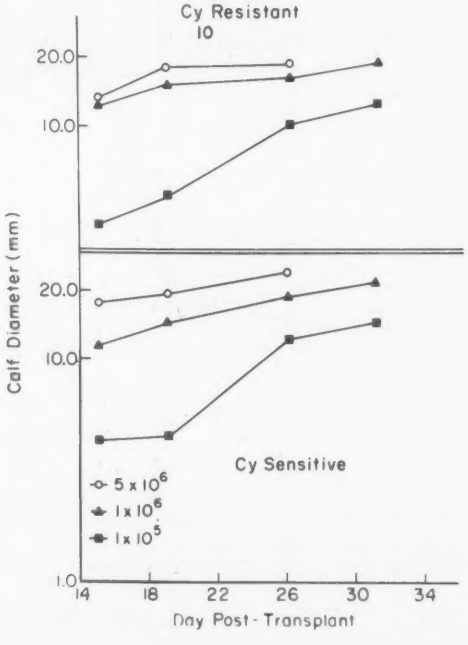

FIGURE 3.—Tumor growth curves indicated by increase in calf diameters from 10 mice following sc injection of various tumor cell numbers. CY-S parent line is shown in the bottom panel for comparison with CY-R_{10} (generation 10) subline in the top panel.

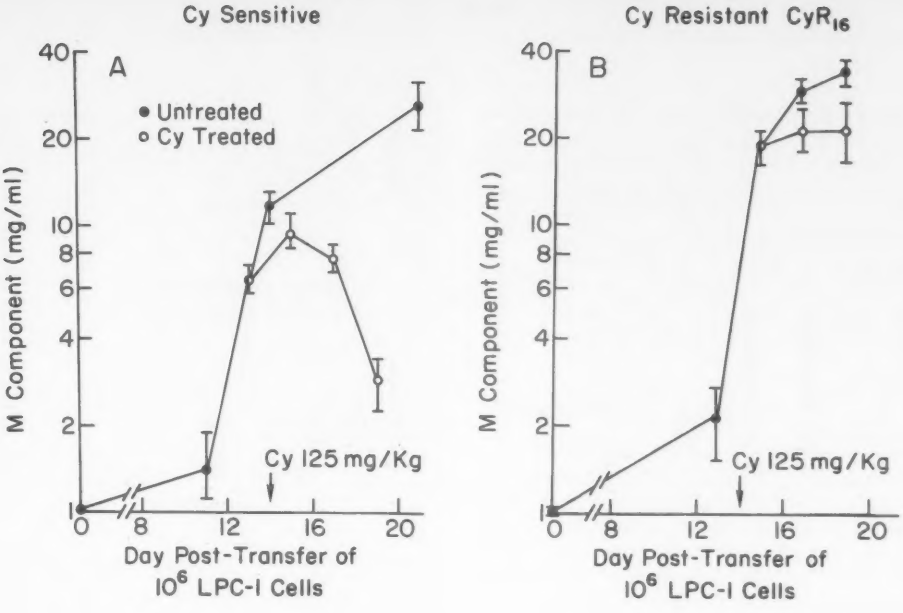

FIGURE 4.—Effect of CY on M component concentration. Tumor growth/regression curves indicated by M component concentration following sc injections of 10^6 tumor cells. This experiment was from generation 16 (CY-R_{16}), before CY resistance was fully developed. As shown in *B*, CY treatment had a temporary modulating effect on M component concentration.

killed mice in 4–5 weeks, with median survival times of 24–26 days. Tumor growth kinetics measured by the emergence and levels of the tumor M component (IgG 2aκ) were very similar in both (CY-S and CY-R) cell lines. The IgG 2aκ M components produced and secreted by CY-S and CY-R cells were identical in their electrophoretic mobility on agarose (9). Plasma M component concentration monitored in both CY-S and CY-R untreated groups of tumor-bearing mice showed the same time-dependent increase during the period of tumor growth (*see* control curves, fig 4). Tumor M component levels of CY-S tumor-bearing mice consistently decreased and were undetectable 2 weeks after CY treatment (125 mg/kg). In contrast, plasma M component concentrations of generation 16 CY-R tumor-bearing mice did not significantly decrease following drug administration (fig 4). The plateau observed after CY treatment was due to the transient regression of tumor in this early generation in the development of the CY-R trait, as shown above in figure 1. In vitro M component synthesis rates of the two cell lines were compared by measuring ^{14}C-leucine incorporation into tumor protein during short-term, 24-hour culture. There was similar time-dependent incorporation of the radiolabeled amino acid into M component, with similar in vitro synthesis rates of 22,000–46,000 molecules of IgG 2aκ/cell/minute.

From a morphologic viewpoint, CY-S and CY-R tumors were very similar in that both exhibited two cell types comprising the tumor: plasmacytoid (33%–52%) and lymphocytoid (48%–67%) and insignificant numbers of granulocytes or other mononuclear cells. Both plasmacytoid and lymphocytoid cell types contained intracellular IgG 2aκ but no detectable cell-surface Ig when examined by indirect immunofluorescence and Sternberger's unlabeled antibody-anti-peroxidase technique (13).

The DNA distributions of CY-S and CY-R tumors were indistinguishable when analyzed by FACS II, which measures the intensity of cellular fluorescence proportional to DNA content (12). The standards used were CRBC and diploid normal mouse spleen lymphocytes which showed peak fluorescence at about channels 20 and 40, respectively (fig 5). Tumor cells stained with propidium iodide re-

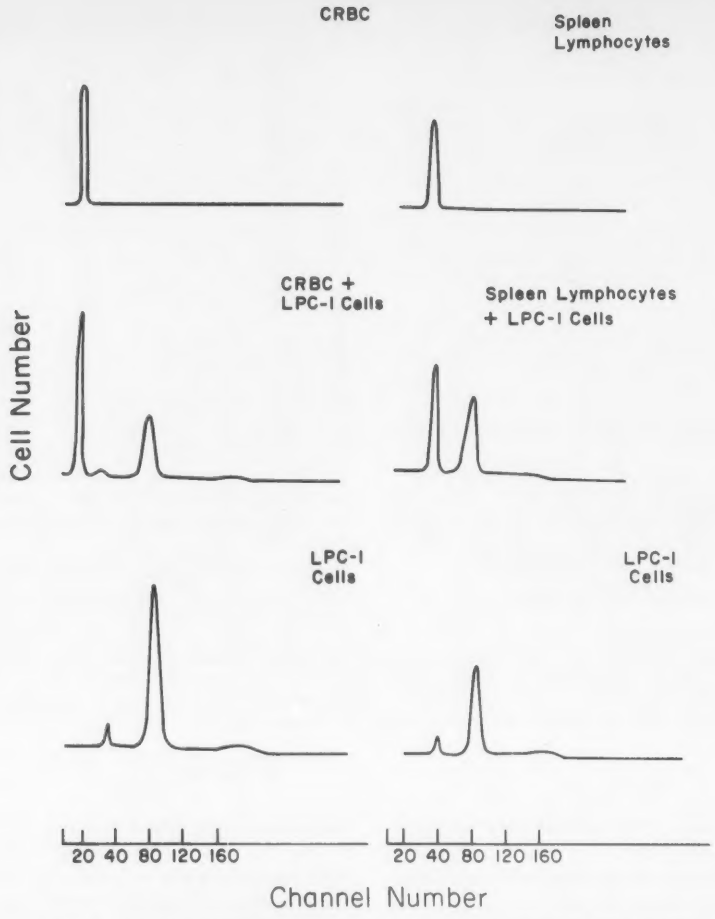

FIGURE 5.—DNA histogram. Representative experiments illustrating the DNA distribution of LPC-1 CY-S or CY-R plasmacytoma cells as determined by FACS II analysis of propidium-stained nuclei. CRBC served as the control for the experiment shown at the left, and normal mouse spleen lymphocytes served as the internal standard for the experiment shown at the right. The middle panel illustrates the results of cell-mixing experiments, and top and bottom panels show the unmixed control cells and the LPC-1 tumor cells, respectively.

vealed three discrete peaks of DNA fluorescence by FACS II analysis (fig 5). The first peak at about channel 40 represents diploid DNA based on normal mouse spleen lymphocytes as an internal control. The second and dominant peak, polyploid (approximately tetraploid), centered around channel 80, and the minor third peak was around channel 160.

To rule out the possibility that solid (sc) CY-R tumor was resistant due to nonspecific host factors affecting tumor growth, such as poor vascular supply, drug inaccessibility, or hypoxia, tumor cells were grown ip as ascites. CY doses curative for CY-S tumors failed to eliminate CY-R tumors grown either sc or in ascitic form. As shown by measurements of tumor M component, CY-R sc and ascites tumors continued to grow following CY treatment (fig 6). These CY-R tumor-bearing mice died by 4–6 weeks.

Experiments were also conducted to determine whether CY-R cells would be more sensitive to the

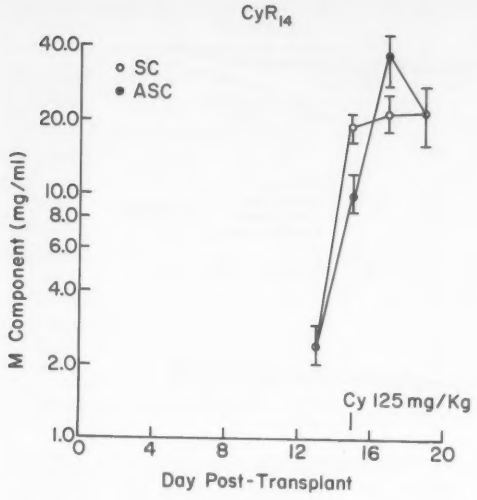

FIGURE 6.—2 groups of 10 mice each received 125 mg/kg of CY ip 14 days after ip and sc transfer of 1×10^6 CY-R_{14} cells (generation 14). Tumor IgG 2aκ M component mean values ± SEM are presented. sc = subcutaneously growing tumor; asc = ascitic tumor.

drug after cell transfer during the "eclipse" phase, the period when tumor cells have not yet been fully established (14). CY was administered following sc or ip transfer of cells, before tumor or ascites and M component were detectable (Days 0–7). CY-R cells continued to grow in mice, as shown by the subsequent increase in tumor size or the development of ascites and the rise in plasma tumor M component. Mice bearing CY-S tumors treated with drug during this time interval (ie, 0–7 days) did not develop detectable tumor ascites, or M component.

Cross-resistance of the CY-R tumors to other alkylating agents structurally similar to CY also was investigated. The response of CY-S or CY-R tumors to CY analogs (ifosfamide and trofosfamide) was tested. Ifosfamide treatment (62.5 or 125 mg/kg of body weight) resulted in regression of established sc CY-S cells and prolongation of survival times from 25 to 48 days (fig 7), whereas CY-R tumors did not respond to ifosfamide at all (fig 7). Trofosfamide induced complete regression of CY-S tumors and prolonged survival times > 60 days (cured) but induced only transient regression of CY-R tumors (fig 7).

Earlier studies indicated that other CY-resistant tumor lines (eg, L1210/CPA leukemia) were not cross-resistant to BCNU or to other functionally and structurally different alkylating agents (7). A similar lack of cross-resistance was observed when the sensitivity of our LPC-1 CY-R tumors to BCNU was investigated. Various doses of the drug were administered ip to mice bearing established sc CY-S or CY-R tumors. A dose of 6 mg/kg was toxic to these mice (20 of 20 died by Day 3), and nonlethal doses of 3, 1.5, and 0.7 mg/kg yielded a narrow dose-response curve as shown in figure 8. Both CY-S and CY-R tumors regressed after treatment with 3 mg/kg as indicated by the decrease in tumor mass (calf diameters) and prolongation of survival time > 60 days (cured; table 2). The lower doses neither reduced tumor mass nor increased survival times (fig 8).

DISCUSSION

In the studies presented here, we described and characterized the first mouse myeloma tumor line (LPC-1) to develop resistance to the alkylating agent CY. Similar to the induction of resistance to other alkylating agents in many murine tumors, development of CY resistance by the LPC-1 plasmacytoma was a slow process requiring progressive drug treatment for several transfer generations (5–8). Once resistance was achieved, the tumor grew unperturbed in the presence of curative doses of the drug. In addition, this CY-R subline has maintained its CY resistance for > 14 transfer generations in the absence of drug exposure. Earlier studies on L1210 leukemia cells showed that CY resistance in the absence of drug could be maintained for > 130 generations (7).

The gradual emergence of CY resistance suggests the gradual evolution of the tumor cell population toward CY resistance by means of the selection pressure of subcurative drug treatment operating on an original tumor cell population heterogeneous for the CY resistance trait. This kind of intrinsic tumor heterogeneity has been suggested by the observable range of variation among cells comprising the tumors, eg, pleomorphic morphology, heterogeneity of biomarker production, selective organ metastasis, and variations in drug sensitivity (1,2,4,5). Tumor heterogeneity with respect to drug sensitivity has been suggested in other cell lines by the emergence of alkylating agent-resistant tumor cells after prolonged low-dose treatment, although a few drug-resistant cell lines (eg, semustine-resistant Lewis lung carcinoma, B16 melanoma, and CY-resistant L1210 leukemia cells) have been obtained after single high-dose administration (7). Skipper and Schabel (3) also developed a CY-resistant Fortner hamster Pla. 1 plasmacytoma with a shorter period of drug exposure. In those studies, the rapid development of drug-resistant cells sug-

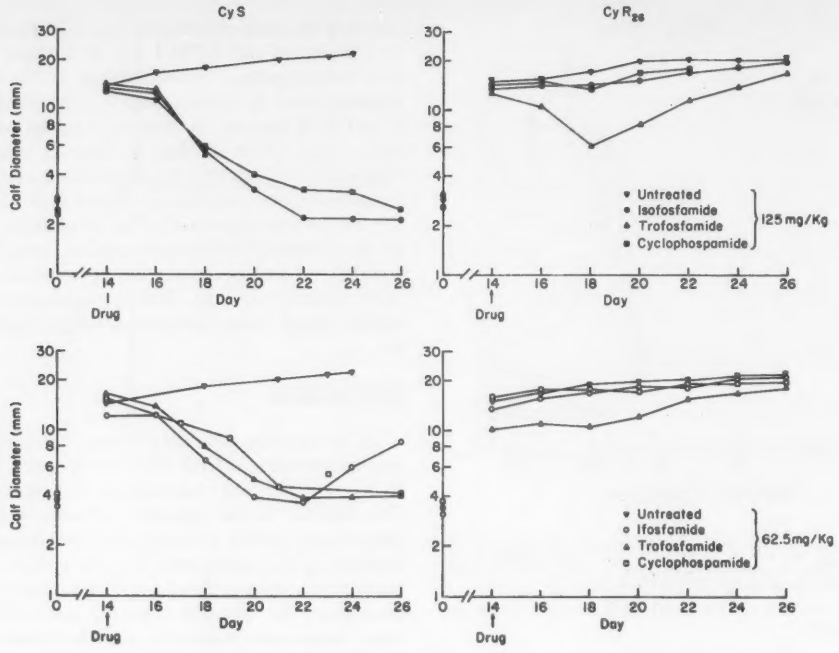

FIGURE 7.—Effect of CY analogs on LPC-1 tumor growth. Mice bearing established sc tumors were treated ip with CY or its analogs, and calf diameter measurements were taken at different time intervals; CY-R_{26} is generation 26.

gests sufficiently high frequency of these cells within certain tumor populations, allowing immediate amplification during high-dose drug exposure.

Other than the difference in their sensitivity to CY, the parent LPC-1 CY-S and the CY-R tumor subline exhibit the same cellular properties with respect to (*a*) growth kinetics, (*b*) synthesis and secretion of tumor IgG 2aκ M component, (*c*) morphology, and (*d*) DNA distribution. These findings are in agreement with earlier studies such as those of Goldenberg (8) and Schabel et al (15) done on other tumor systems where drug-sensitive and -resistant cell lines exhibit similar growth rates and similar cytologic and biochemical features.

FACS II analysis indicated identical DNA distribution (fig 5) of the parent CY-S and CY-R tumor cells, suggesting similar ploidy. Previous studies also failed to demonstrate significant differences in the ploidy as well as the karyotype of alkylating agent-sensitive and -resistant tumor cells (8,16,17). Goldenberg (8) earlier reported increased percentage of tetraploid cells in association with mechlorethamine resistance, but considerable overlap was also noted between drug sensitivity and polyploidy. Chromosome analyses on our CY-R and CY-S lines have not yet been performed.

Other studies have demonstrated that structural differences or similarities in alkylating agents may predict for biologic effects and for cross-resistance. We have examined the ability of our CY-R tumor cell line to respond to closely related drugs (the CY analogs ifosfamide and trofosfamide), and another, structurally distinct, alkylating agent (BCNU). Ifosfamide differs from the parent compound in that its second chloroethyl group is attached to the ring nitrogen whereas trofosfamide has a third additional chloroethyl group in this position. The CY-R tumor was also resistant to these analogs (fig 7, table 2), indicating that changing the position and number of chloroethyl groups could not overcome the drug resistance at the doses used (62.5 and 125 mg/kg). It is interesting that trofosfamide treatment extended survival times of CY-R tumor-bearing mice more than CY did, since this analog has two additional chloroethyl groups.

When a structurally different alkylating agent (BCNU) was used, the CY-R tumor behaved like its CY-S parent; both tumors responded, and both

TABLE 2.—Response comparison of LPC-1 CY-S and CY-R to CY analogs

Treatment and dose (mg/kg)	Median survival time (days) of mice bearing—	
	LPC-1 CY-S	LPC-1 CY-R
Untreated	25	25
CY		
62.5	> 60	25
125.0	> 60	25
Ifosfamide		
62.5	48	28
125.0	48	28
Trofosfamide		
62.5	> 60	20
125.0	*	39

*All animals died by Day 18.

CY-S and CY-R tumor-bearing mice were cured (ie, survival > 60 days). However, the murine host (BALB/c mice) failed to tolerate higher drug doses, and lethal toxicity was observed at twice the curative dose. Thus, the dose-response curve analyzed was quite narrow (fig 8).

The BALB/c mouse LPC-1 plasmacytoma has been in continuous, serial in vivo passage for more than two decades. Over this period, it has exhibited a stable growth pattern and the continuous production of a tumor-specific IgG 2aκ M component (9,14,16). In the studies presented here, we report the development of a CY-R LPC-1 plasmacytoma which, by all criteria studied, is indistinguishable from the CY-S parent line. For the first time, a mouse myeloma model is available that will permit the analysis of cellular and molecular changes which could account for the development of tolerance of myeloma cells to alkylating agents. These special features of our model offer promising investigational approaches to the biology of the development of resistance to alkylating agents.

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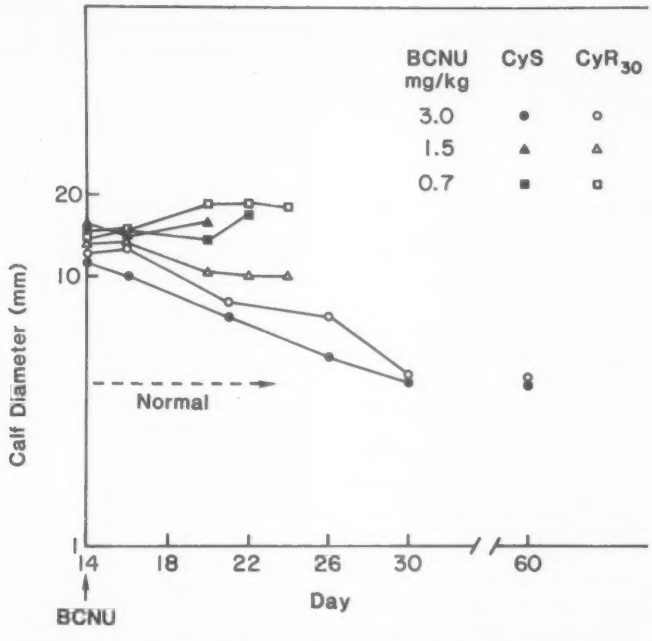

FIGURE 8.—Dose-response curve of LPC-1 plasmacytoma to BCNU. Mice bearing sc CY-S or CY-R tumors were injected ip with varying doses of BCNU, and calf diameters were monitored at different time intervals; CY-R_{30} is generation 30.

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Modification of Radiation Damage in Rat Spinal Cord by Mitotane[1,2]

Arvin S. Glicksman,* Sarah F. Bliven, and John T. Leith[3]

SUMMARY

Modification of the paralytic response in rats after 6-MV photon irradiation of the spinal cord with either single or split exposures (two equal fractions given in a 24-hour period) by mitotane was investigated. Mitotane was administered as a suspension in physiologic saline (300 mg/kg/day) for either 5 days prior to or 5 days after irradiation. For rats receiving split doses of 6-MV photons, either the last two doses of mitotane were given 2 hours prior to each radiation fraction or mitotane was begun 2 hours after the second fraction and continued for 5 days. The data to 6 months after irradiation indicate that, in rats given mitotane for 5 days *prior* to single-dose photon irradiation, the paralytic response (as defined by the dose needed to produce paralysis in 50% of the irradiated groups of rats) was enhanced by a dose-enhancement factor (DEF) of 1.40. The DEF in the group of rats given mitotane *after* single doses of 6-MV photons was 1.15. In the split-dose irradiation experiments, the DEF for the groups of rats given mitotane *prior to* each radiation fraction was 1.36; while the DEF for the group of rats receiving mitotane beginning *after* the second fraction was 1.18. These data indicate that mitotane can potentiate the effects of 6-MV photon irradiation to the central nervous system, with mitotane administered prior to irradiation being the most effective sequence.

[Cancer Treat Rep 66:1545–1547, 1982]

Mitotane (*O,P'*-DDD) is an isomer of the insecticide tetrachlorodiphenylethane (DDD). This agent has been used in the palliative treatment of inoperable adrenocortical carcinoma. Bergenstal et al (1) and Lubitz et al (2) have described the antitumor activity of this compound, and it is used in the treatment of metastatic disease (3,4) and Cushing's syndrome (5,6). Mitotane has been shown to induce severe adrenal cortical atrophy when given to dogs (7), an effect that appears to be mediated through effects on the mitochondria of the adrenal cortical cells, leading to focal lesions in the fascicular and reticular zones of the adrenal cortex (8). Concomitantly, secretion of steroids by the adrenal gland is reduced (9). The drug appears to be stored primarily in fatty tissues throughout the body (10) and can be detected in the blood for several months after administration is discontinued. While the liver appears to metabolize relatively small amounts of the drug, the bile appears to be a significant organ of elimination (as the ethene or acetate derivatives of mitotane).

A large number of patients treated with the drug have exhibited central nervous system (CNS) side effects, including lethargy, sedation, vertigo, and dizziness. Long-term use may lead to overt brain damage (2,10). As this agent has been shown to produce damage in the CNS, we wanted to determine whether it would interact with damage to the CNS produced by sparsely ionizing radiation. We irradiated the thoracolumbar regions of the verte-

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bral columns of rats with graded doses of either single or split (two equal fractions) doses of 6-MV photons. This model for radiation-induced myelopathy has been used by us for investigation of high linear energy transfer (LET) radiation on the spinal cord (11) and for investigation of the potentiation of low LET ionizing radiation on the spinal cord by whole-body hyperthermia (12). The theoretic implications of data derived from this normal tissue model system have been published (13).

MATERIALS AND METHODS

The rats used were adult CDF females obtained from the Charles River Breeding Laboratories, Wilmington, MA. They were housed in large, wire, hanging cages and were given food (Purina Lab Chow, Fort Wayne, IN) and water ad libitum. Rats weighed about 180 g at the time of treatments.

For irradiation, rats were exposed with a 6-MV Siemanns linear accelerator. Rats were anesthetized with sodium pentobarbital ip (50 mg/kg) and were exposed transversely to the thoracolumbar (T12-L1) region of the vertebral column. A source-to-skin distance of 50 cm was used, with the dose calculated at the center of the vertebral column. The field was collimated so that a 2-cm length of vertebral column was exposed, and a bolus of beeswax was placed over the dorsal region of the rat so that the incident photon beam encountered a constant amount of material before reaching the position of the spinal cord. The dose rate was about 5 Gy/minute.

The mitotane (Bristol Laboratories, Syracuse, NY) was prepared from tablet form. Administration of this agent in humans is by mouth, over a long period of time at relatively high doses. The tablets (500 mg each) were initially crushed to a fine powder using mortar and pestle. Each tablet was suspended in 16.6 ml of physiologic saline to give a drug concentration of 30 mg/ml. The rats were given 300 mg/kg of mitotane (about 1.8 ml of suspension) per administration. This drug suspension was administered daily for 5 days by stomach tube to rats that had been lightly anesthetized by methoxyflurane (Metofane; Pitman-Moore, Inc, Washington Crossing, NJ). To check on the reproducibility of our administration, replicate samples of drug suspension were withdrawn from the stirred solution and evaporated to dryness; the remaining powder was weighed. It was determined that each dose administered to the rats was accurate to within 1.03% (95% confidence limits).

For the combined photon irradiation and drug experiments, the protocol shown in table 1 was used. There were five dose levels (including no irradiation for controls) used per treatment regimen, with eight rats per dose level. These combined treatments were given in two sequences (ie, drug either before or after irradiation) and with two dose schedules (single and split doses of irradiation) and were intended to determine whether mitotane had any potentiating effect on spinal cord damage, what the effect of sequencing was, and whether the drug had any effect on repair of subeffective radiation injury in the spinal cord.

RESULTS AND DISCUSSION

We determined the dose needed to effect paralysis in 50% of the irradiated groups of rats, with and without mitotane administration. The endpoint chosen was equivalent to induction of a definite but mild impairment of hindlimb kick, extensor and flexor

TABLE 1.—Treatment schedule*

Treatment	Day of treatment										
	1	2	3	4	5	6	7	8	9	10	11
Single doses											
R alone						R					
M + R	M	M	M	M	M	R					
R + M						R	M	M	M	M	M
Split doses											
R alone						R_1	R_2				
M + R		M	M	M	M	M + R_1	M + R_2				
R + M					R_1	R_2 + M	M	M	M	M	M

*R = radiation treatment. Single-dose radiation levels were 21.25, 23.75, 26.25, and 28.75 Gy. R_1 and R_2 denote equal split-dose radiation treatments. Split exposure total doses were 29.50, 32.50, 35.50, and 38.50 Gy. Mitotane (M) was administered at a dose of 300 mg/kg. In M + R_1 and M + R_2 treatments, mitotane was given 2 hrs prior to each radiation factor; in R_2 + M treatment, mitotane was given 2 hrs after second radiation treatment.

TABLE 2.—Estimated ED50 and DEFs after spinal cord irradiation in the rat

Treatment*	ED50 (Gy)†	DEF‡
Single doses		
R alone	28.50 ± 1.5	—
M + R	20.50 ± 1.3	1.39 ± 0.11
R + M	24.50 ± 1.2	1.16 ± 0.08
Split doses		
R alone	35.50 ± 2.1	—
M + R	26.00 ± 1.8	1.36 ± 0.12
R + M	30.00 ± 2.0	1.18 ± 0.10

*R = radiation treatment (6-MV photons); M = mitotane.
†Values = mean ± SE (95% confidence limits).
‡Values = DEF ± SE. Errors are propagated 95% confidence limits.

activities, based on a subjective grading scale that we have used in other studies (11). These are early data, up to 6 months after irradiation. To date, we have not lost any animals in our study.

The doses of radiation needed to produce 50% myelopathy in rats as a function of treatment schedule are shown in table 2; the dose-enhancement factors (DEFs) found by comparing these ED50 values are also listed. Error limits on the ED50 and DEF values were calculated by methods previously described (11). For both the single and split photon irradiation schedules, mitotane given prior to irradiation yields DEFs of 1.36–1.39; for mitotane given after photon irradiation, the DEFs are 1.16–1.18. Therefore, mitotane is more effective in potentiating photon injury to the spinal cord when present prior to irradiation. Also, if the fractional amount of radiation damage repaired is calculated (11), the repair values are similar for all situations (ie, photon irradiation alone or photon irradiation plus mitotane given either prior to or after irradiation). This suggests that mitotane is acting as a pure sensitizer and is not acting through inhibition of the repair of subeffective radiation damage in the spinal cord.

Mitotane has been used periodically in the management of adrenal cortical carcinoma, particularly when it has been associated with endocrine-secreting syndromes (14). It has been effective in many of these situations without significantly affecting tumor control or survival (15). The drug also has a role in the management of Cushing's syndrome and may be used in patients who have previously been treated with radiotherapy. These early data indicate that mitotane does interact with damage to the CNS produced by sparsely ionizing radiation and enhances the radiation effect. Therefore, the inadvertent combined use of the drug and radiation may result in an unexpected CNS injury, which neither the drug alone nor the radiotherapy alone would have produced. On the other hand, utilization of this sensitization effect for potential therapeutic gain is a possibility that needs further investigation.

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Treatment of Neoplastic Meningitis With Intraventricular Thiotepa and Methotrexate[1,2,3]

Brief Reports

Donald L. Trump,* Stuart A. Grossman, George Thompson, Kenneth Murray, and Moody Wharam[4]

SUMMARY

Twenty-five patients with involvement of the meninges by disseminated cancers were treated weekly with intraventricular thiotepa (10 mg) and methotrexate (10 mg) and with radiation therapy. Twenty-two of the 25 patients were clinically improved or stable. Malignant cells were cleared from the cerebrospinal fluid in 76% of the patients. Myelosuppression was the dose-limiting toxic effect, and neurotoxicity occurred in three patients. Combination chemotherapy employing thiotepa and methotrexate is a safe and effective treatment for neoplastic meningitis.

[Cancer Treat Rep 66:1549–1551, 1982]

Increased attention has been focused recently on leptomeningeal involvement by disseminated cancer (neoplastic meningitis) (1–4). The prognosis for such patients is poor, with a median survival of 1½–4 months (5,6). Recommended treatment for neoplastic meningitis includes radiation therapy to symptomatic regions of the neuroaxis and intrathecal cytotoxic chemotherapy given through an intraventricular catheter (7).

Improved treatment of neoplastic meningitis may be attained by developing new intrathecal drugs and drug combinations. This report describes a phase II evaluation of combination intrathecal chemotherapy employing thiotepa and methotrexate.

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[3]Presented in part at the Meeting of the American Association of Cancer Research, Washington, DC, April 1981.

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MATERIALS AND METHODS

Twenty-five patients were treated for neoplastic meningitis. Twenty-three patients had a positive cerebrospinal fluid (CSF) cytology, and two patients had disseminated cancer, multifocal neurologic defects, parameningeal foci of cancer, and an abnormal CSF (table 1). Panmyelography and cerebral computer-assisted tomographic scans were performed in each patient.

All patients received radiation therapy (2500–3000 rad/10 days) to symptomatic areas of the neuroaxis. Intrathecal chemotherapy was begun within 7 days of the recognition of neoplastic meningitis and was administered through a subcutaneous reservoir and intraventricular catheter. Treatment consisted of the weekly administration of thiotepa (10 mg in 10 ml of buffered diluent on Day 1) and methotrexate (10 mg in 10 ml of buffered diluent on Day 4). Weekly therapy was continued for either 8 or 12 weeks, depending on the rapidity of CSF and clinical re-

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total in study	25
Sex	
Female	18
Male	7
Median age in yrs: 50 (range, 22–76)	
Primary diagnosis	
Breast cancer	16 (64)
Lung cancer	2 (8)
Lymphoma/leukemia	4 (16)
Other cancers	3 (12)
Parenchymal brain metastasis	10 (40
Epidural spinal cord compression	10 (40)
Positive CSF cytology	
Lumbar	23 (92)
Ventricular	12 (48)
Active, progressive cancer outside the CNS	15 (60)

sponse. After induction therapy, responding patients were treated for 8 or 9 additional months, with 1 week of thiotepa and methotrexate monthly. If myelosuppression or stomatitis occurred, oral leucovorin factor was administered after intrathecal methotrexate. All 25 patients received at least 2 weeks of intrathecal therapy.

Required treatment for systemic cancer was administered concurrently with therapy for neoplastic meningitis. In 18 patients large doses of corticosteroids were administered for the first 14–35 days of treatment.

RESULTS

Clinical Response

Three patients (12%) experienced progressive deterioration in neurologic signs and symptoms during therapy. Thirteen patients (52%) were neurologically stable for at least 6 weeks following initiation of therapy. Five patients had definite partial improvement in neurologic signs and symptoms, and in four patients (16%) complete resolution of all neurologic signs and symptoms occurred. Median survival was 23 weeks for all patients measured from Day 1 of intrathecal chemotherapy.

Laboratory Parameters

In 13 of 17 (76%) evaluable patients, lumbar CSF was cleared of cancer cells for at least 1 month. In eight of 12 (66%) evaluable patients, malignant cells were eradicated from the ventricular CSF for > 1 month.

Hypoglycorrhachia was reversed in all nine patients in whom it was present in the initial lumbar CSF sample. Pleocytosis was present in lumbar CSF in 16 patients and in the ventricular CSF in eight patients and resolved in both sites in all patients.

Toxicity

Myelosuppression was the major toxic effect of this treatment. Nineteen (76%) patients developed leukopenia of < 2500 wbcs/mm^3 and in nine (36%) it was profound (< 1000 wbcs/mm^3). In 14 (56%) patients thrombocytopenia occurred (< 100,000 cells/mm^3); asymptomatic platelet counts < 10,000/mm^3 were seen in four. One death was due in part to granulocytopenia.

The myelosuppression experienced by these patients was attributable to concomitant systemic cytotoxic therapy and the irradiation of large volumes of bone marrow. Six patients received only intrathecal chemotherapy with or without cranial irradiation and none developed platelet counts < 100,000/mm^3. Two patients developed wbc counts < 2500/mm^3, and no patients had a wbc count < 1000/mm^3. All other patients received extensive radiation therapy (13 patients) and/or concomitant myelosuppressive therapy (nine patients).

CSF infections occurred in four patients (8). One infection occurred in a patient with a wbc count < 1000/mm^3. All infections were controlled with antibiotics and removal of the subcutaneous reservoir and intraventricular catheter.

Treatment-related neurotoxicity, characterized by memory loss and a broad-based shuffling gait, occurred in three patients. In two patients these findings partially resolved when intrathecal therapy was stopped, while in the third no improvement occurred.

Sixteen of the 25 patients have died. Progressive systemic cancer was the cause of death in six (38%) patients, while in another six (38%) death was due to a combination of progressive systemic and CNS malignancy. Toxicity related to treatment of neoplastic meningitis contributed to the death of two patients. In four (25%) patients death was due to uncontrolled CNS disease alone.

DISCUSSION

Gutin et al (9,10) have reported phase I–II trials indicating the safety and efficacy of intrathecal thiotepa in patients with refractory meningeal lymphoma and leukemia. Myelosuppression and neurotoxicity were noted in their trial. These data suggested the feasibility of combining thiotepa with methotrexate to investigate combination intrathecal chemotherapy.

Our study demonstrates that such combination therapy is feasible and effective. The chemotherapy itself caused only mild toxic effects. The major toxic effect in our series was myelosuppression, which was primarily due to the simultaneous administration of systemic chemotherapy and radiation therapy.

The role of intraventricular versus intralumbar administration of chemotherapy, the benefit of local radiation therapy, and the efficacy of thiotepa and methotrexate compared to "standard" intrathecal chemotherapy remain to be determined in neoplastic meningitis. Our data indicate that combination intrathecal chemotherapy utilizing an alkylating agent and an antimetabolite is safe and yields encouraging therapeutic results. Prospective trials comparing thiotepa and methotrexate to single-agent therapy will be required to determine the relative merits of these treatment plans.

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Sequential Methotrexate and 5-FU in the Treatment of Colorectal Cancer[1]

Brief Reports

B. Weinerman,* B. Schacter, H. Schipper, D. Bowman, and M. Levitt[2]

SUMMARY

Administration of methotrexate (MTX), 5-FU, and leucovorin rescue in 29 patients with metastatic colorectal cancer produced an objective response rate of 41.7%, with two complete remissions. Two patients developed fatal toxic effects and three others had severe toxic effects. This trial, however, supports the evidence for the synergistic effect of this drug combination.

[Cancer Treat Rep 66:1553–1555, 1982]

There are data suggesting that the combination of methotrexate (MTX) and 5-FU may be synergistic in the treatment of cancers (1–4). Theoretically, this is accomplished by shunting more 5-FU (5–8) into RNA production, where it may act more effectively. Furthermore, other data suggest that this synergistic effect may not be present for bone marrow cells, thereby increasing the therapeutic index of the agents (3). In view of these reports, we initiated a phase II trial to determine the response rate and the toxicity of MTX, 5-FU, and leucovorin rescue (LR) in the treatment of metastatic colorectal carcinoma with measurable disease.

METHODS

All patients, whether previously treated or not, were admitted to the trial if they had a creatinine clearance > 60 ml/minute, an Eastern Cooperative Oncology Group (ECOG) performance status < 4, and measurable disease.

Table 1 illustrates the chemotherapy protocol. All patients were well-hydrated and were alkalized with $NaHCO_3$ to keep the urine at a pH > 6.5 throughout the treatment.

No attempt was made to guide the total number of treatments given, although it was recommended that treatments be continued (minimum, two) until progressive disease intervened. Treatment was administered every 21–28 days if the wbc count was > 4000/mm^3, the platelet count was > 100,000/mm^3, and the creatinine clearance was > 60 ml/minute.

MTX levels were determined in several patients by the ^{125}I-MTX radioimmunoassay.

Response evaluation criteria included: *complete response (CR)*—complete tumor disappearance; *partial response (PR)*—a ≥ 50% reduction in tumor volume; *stable disease*—no change or < 50% reduction in tumor volume; and *progressive disease*.

RESULTS

When all 29 patients were included in the analysis, there were two patients (6.8%) with CR, eight (27.6%) with PR, and 11 (38%) with progressive disease. If only those patients who received two or more treatments were analyzed the CR rate was 8.3% and the PR rate was 33.3%; 33.3% of the patients had stable disease; and 25% had progressive disease. The median duration of remission was 4 months, and the median duration of stable disease was 5 months.

Five patients had one treatment, six had two, six had three, five had four, and seven had five or more treatments. There were a total of 100 treatments in 29 patients. Toxicity was noteworthy: 11.1% of the patients had a wbc count nadir < 1500/mm^3, and similarly, 11.1% had a platelet count nadir < 50,000/mm^3; 22.2% developed mucositis; 13.3% developed dermatitis; and 8.8% (four patients) had renal complications. Two patients died with severe dermatitis, mucositis, and leukopenia, with subsequent infection. Responding patients and those with stable disease survived significantly longer ($P < 0.001$) than nonresponding patients (fig 1). There was no significant survival difference between responders and those with stable disease (fig 1).

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TABLE 1.—Protocol*

Time	Drug (dose)
Hour	
0	D5W† ½ normal saline (150 ml/hr) with $NaHCO_3$ (3 g) orally every 6 hrs for 30 hrs
3	MTX (20 mg/kg in 500 ml of D5W) infused continuously for 4 hrs
7	5-FU (600 mg/m²) iv bolus. D5W ½ normal saline (150 ml/hr) until the end of therapy at Hour 28
16	LR (10 mg/m² iv)
22	LR (10 mg/m² iv)
28	LR (10 mg/m² iv)
34	LR (10 mg/m² iv)

*Study criteria were normal BUN and creatinine, creatinine clearance > 60 ml/min, and ECOG performance status < 4. Treatments were repeated every 21–28 days.

†D5W = 5% dextrose in water.

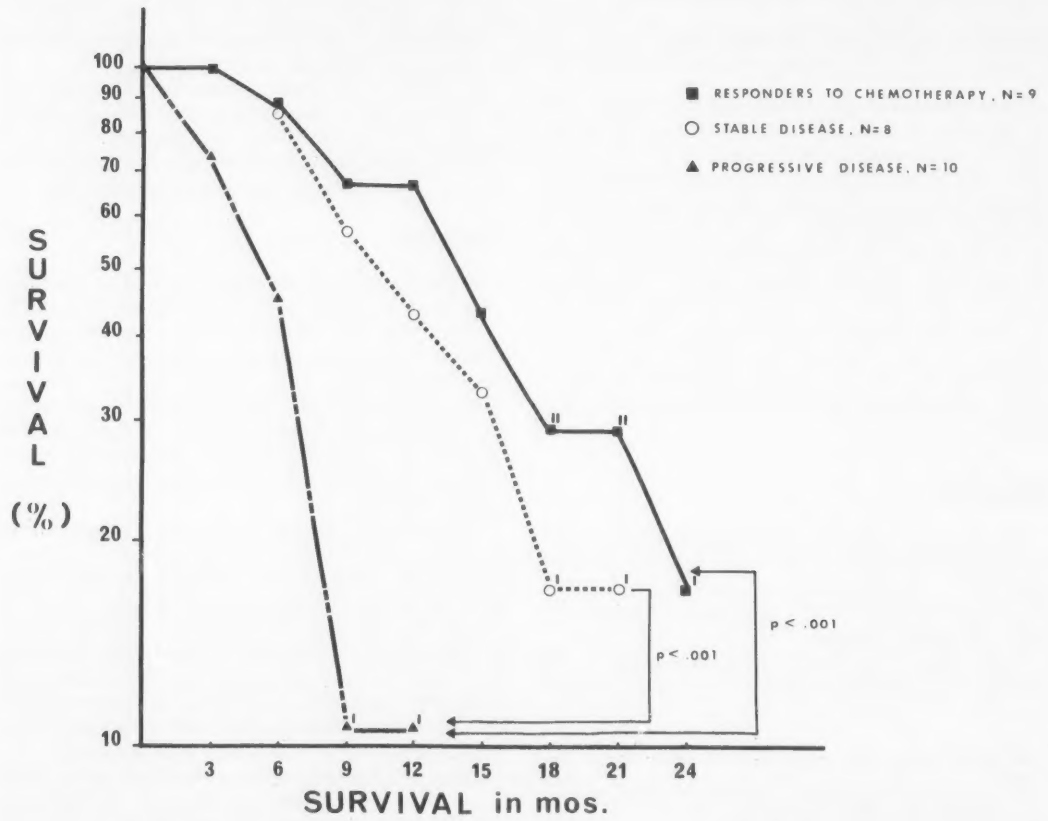

FIGURE 1.—Survival curve: metastatic colorectal cancer.

MTX levels were not measured in the five patients who developed severe toxic effects. The MTX levels that were determined were very low.

CONCLUSIONS

It appears that the combination of MTX, 5-FU, and LR as given in the series had a relatively high response rate (41.7%). This is supportive evidence for the synergistic effect of this combination. However, the toxicity was often severe and unpredictable. Two of the patients developed severe toxic effects during the first treatment, and the three others developed them in a subsequent treatment, despite adequate renal function and appropriate LR. MTX levels were not measured at the beginning of each treatment cycle, so that it is possible that MTX or 5-FU levels were still present from earlier treatments, although at least 3 weeks had elapsed between the treatment cycles. Remission durations were not long (4–5 months), but this trial was not rigidly designed to look at remission duration, so continued treatment in some of the patients may have lengthened remission and survival durations.

The utilization of MTX, 5-FU, and LR merits more exploration. Modifications in scheduling to increase response rates and lessen toxic effects must be tried. Longer treatment programs may maintain remissions longer. Care must be taken, however, in view of the toxicity of the program. Measurement of serum MTX levels appears to be mandatory in the attempt to elucidate toxicity mechanisms.

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CCNU, Vincristine, Methotrexate, and Procarbazine Treatment of Relapsed Small Cell Lung Carcinoma[1,2]

Brief Reports

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SUMMARY

Thirty-two patients with refractory relapsed small cell carcinoma of the lung (SCCL) were treated with a combination of CCNU (lomustine), vincristine, methotrexate, and procarbazine (COMP); 29 were evaluable for response. Nine patients (31%) had responses: five complete responses (CR) and four partial responses. Patients with CR had a median survival of 11 months (range, 5½–14½) from the start of COMP. Patients with less than CR had a median survival of approximately 3 months (range, < 1–7). The comparison of CR versus less than CR is significant (P = 0.003). Patients achieving CR usually had limited disease and four of the five who achieved CR survived > 6 months. The regimen was well-tolerated, but myelosuppression was seen in all patients. COMP appears to be a useful combination in patients with relapsed SCCL. Aggressive retreatment should be considered in relapsed patients with this disease since some may achieve a second CR, with the associated potential survival benefit.

[Cancer Treat Rep 66:1557–1559, 1982]

Despite recent improvements in the treatment of small cell lung carcinoma (SCCL) (1–3), most patients still relapse after successful primary treatment (4,5). With rare exceptions, patients who relapse or have refractory disease have rapidly progressive disease, with short survival irrespective of subsequent treatment (3,6,7). Hence, the current study was undertaken to evaluate the combination of the active agents CCNU (lomustine), vincristine, methotrexate, and procarbazine (8) in patients with recurrent or refractory disease previously treated with cyclophosphamide, doxorubicin, and etoposide.

MATERIALS AND METHODS

All patients who relapsed or had progressive disease while receiving primary treatment were eligible for study. All patients had previously received a combination of cyclophosphamide, doxorubicin, and etoposide (CAVP-16) (2) as initial therapy and had histologic proof of SCCL, recovery from the toxic effects of prior therapy, normal renal and hepatic functions (serum creatinine < 1.5 mg/dl and serum bilirubin < 1.5 mg/dl), and total wbc count > 3000/μl and platelet count > 100,000/μl.

All patients had a complete restaging evaluation including: physical examination, cbc and differential, serum chemistry, chest roentgenogram, scintiscans of bone and liver, computerized tomographic brain scan, and bilateral bone marrow aspirates and biopsies either at relapse or prior to protocol entry. Performance status was scored according to the Karnofsky scale, and extent of disease was scored according to the usual criteria. All patients evaluable for response had at least one measurable indicator lesion at the start of the protocol.

Chemotherapy on a 42-day cycle consisted of CCNU (75 mg/m²) orally on Day 1; vincristine (1.4 mg/m²; maximum dose, 2 mg) iv and methotrexate (40 mg/m²) iv on Days 1 and 21; and procarbazine (75 mg/m²/day) orally on Days 1–5 and 21–25 (COMP). If neutropenia or thrombocytopenia occurred at the time of re-treatment, therapy was usually delayed for 1 week, doses were reduced, or both. However, in instances of bone marrow involvement, no modifications were made. All patients were treated until relapse or disease progression, and there was followup of all patients until death. Radiation therapy was utilized during the course of COMP chemo-

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therapy only for intracranial metastasis. Standard response criteria were employed (2). Durations of response and survival were measured from the start of COMP therapy.

RESULTS

Three of 32 patients entered in the study were considered inevaluable since they did not fulfill protocol entry requirements. Characteristics of the 29 evaluable patients are shown in table 1. When staged for primary therapy, 15 of the 29 patients had had limited disease and 14 had had extensive disease. At the time of COMP (relapse) therapy, eight patients had only local relapse or progression (limited disease) and 21 had extensive disease relapse.

Response

The overall complete and partial response rate (CR + PR) was 31%, with five patients (17%) achieving CR and four (14%) achieving PR. Eighteen patients did not respond to this regimen, and two had disease stabilization for approximately 2 months. Four of the five patients achieving CR had limited disease. None had received prior chest irradiation and three of the four patients with limited disease had previously achieved CR. Among the patients with extensive disease, one had CR and three had PR. Attainment of CR correlated with limited extent of disease ($P = 0.003$) but not with age, performance status, or duration or magnitude of response to previous chemotherapy. The median duration of response for the five patients achieving CR was 7.5 months (range, 3–10) compared to the median response of 3 months for those patients who achieved PR. Response was usually observed by the end of the first course, and responding patients received two to seven courses of chemotherapy. Twelve of the 18 nonresponding patients received only one course of COMP before showing disease progression. For these 29 patients with relapsed disease, there was no apparent correlation between the durations of the response to therapy with CAVP-16 and to the COMP treatment.

TABLE 1.—Characteristics of patients receiving COMP

Characteristic	No. of patients
Evaluable for—	
Response	29
Toxic effects	28
Median age in yrs (range): 60 (40–69)	
Sex	
Males	20
Females	9
Limited disease	8
Performance status	
80	4
70–80	11
70	14
Prior CAVP-16	29
Prior CNS radiotherapy	6
Prior non-CNS radiotherapy	10
Median duration in mos of response to prior therapy (range): 7.7 (2–20)	

Survival

Overall survival from initiation of COMP until death ranged from < 1 to 15 months. Patients with limited disease had a median survival of 6.5 months compared to 3 months for patients with extensive disease ($P = 0.003$). Patients who achieved CR had a median survival of 11 months (range, 5.8–15), while those who achieved PR had a median survival of 4 months (range, 3–6). Those with less than PR had a median survival of 2.5 months (range, < 1–7). Patients with CR survived longer than those with less than CR ($P = 0.003$). Four of the five patients achieving CR had survival > 6 months. Pretreatment performance status also correlated directly with survival ($P = 0.001$).

Toxicity

In the 28 patients evaluable for toxic effects, myelosuppression was most prominent. Dose reduction of myelosuppressive agents, usually to 50% of the scheduled dose, was required in nine of 17 patients receiving more than one course of chemotherapy. The median wbc count nadir of each patient during therapy was 2400/μl, and six patients had nadirs ≤ 1000/μl. The median platelet count nadir was 61,000/μl, and six patients had nadirs ≤ 20,000/μl. Patients with platelet counts < 20,000/μl received prophylactic platelet transfusions. Only one of 14 patients with platelet counts < 50,000/μl had a serious bleeding episode.

Other side effects included alopecia and nausea and vomiting. Mucositis was noted in one patient; one developed bilateral foot drop; and two, who had received radiation to the midchest and upper chest, experienced unilateral vocal cord paralysis. No hepatic or renal toxicity was noted. There were no drug-related deaths.

DISCUSSION

COMP was studied in 29 patients who had previously received chemotherapy, 13 of whom had also

received radiotherapy. Contrary to the usual low response rate in patients with relapsed or progressive SCCL, COMP produced an overall response rate of 31%, with 17% CR, 14% PR, and survival > 6 months for five patients. The presence of limited relapse figured largely in these favorable results. Among the eight patients with limited relapse, four achieved CR. The overall median duration of survival for patients with limited relapse was 6.5 months, more than twice that seen in patients with extensive disease. The median survival for patients with CR was 11 months compared to the 3-month median survival in patients not achieving CR. This study suggests that COMP represents one therapeutic option for relapsed SCCL and that it is non-cross-resistant to the primary combination of cyclophosphamide, doxorubicin, and etoposide. At the time of initiation of this study, procarbazine was considered to be active in SCCL (8). However, since more recent data (9) have indicated that this drug may not be active, its inclusion in such future salvage regimens may be subject to reconsideration.

The results of this study suggest that for patients who relapse with limited disease and good performance status, survival may be significantly augmented with the use of the active second-line treatment. This may be of importance in patients for whom new drug investigation is not feasible. Furthermore, the identification of active second-line treatments may produce more active first-line treatments or regimens useful for alternating chemotherapy.

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Neurologic and Cardiac Toxicity Following Iv Application of Methotrexate[1]

Brief Reports

Adrian B. Gasser,[2,*] Marcel Tièche,[3] and Kurt W. Brunner[2]

SUMMARY

The case of a 61-year-old female patient with metastatic breast cancer is reported. She developed neurologic symptoms and supraventricular extrasystoles when she received iv methotrexate. These symptoms were reproducible and were not found with other drugs. The cause for this event is not clear.

[Cancer Treat Rep 66:1561–1562, 1982]

CASE REPORT

We have recently observed a patient who developed neurologic symptoms and cardiac arrhythmia following iv methotrexate (MTX) administration in conventional doses during treatment of metastatic breast carcinoma.

This 61-year-old female had had allergic bronchial asthma since 1968. She was also allergic to penicillin. In 1978 she underwent a mastectomy for an infiltrating ductal carcinoma of the left breast. The clinical stage was indicated as $T_2N_0M_0$. Postoperative radiotherapy was given.

The patient was hospitalized on March 23, 1981, reporting backache. The radiologic examination revealed metastases in the thoracic and lumbar spine, the pelvis, and the right femur. No other metastases were found. The physical examination was normal except for skeletal pain on percussion. The hematologic values were normal except for an unexplained eosinophilia found on hospital admission and persisting until May 23, when it disappeared spontaneously. It ranged from 12% to 16%, with a calculated absolute eosinophil count of 180–918/μl. Serum creatinine was normal (1.1 mg/dl), as was serum calcium (10 mg/dl). The alkaline phosphatase was elevated at 561 units/liter (normal up to 170). SGOT was also slightly increased at 42 units/liter as was SGPT at 73 units/liter. The ECG showed a sinus rhythm. The P-Q interval was shortened (0.11 secs) due to a delta wave in leads II, avF, V2, and V3. A left axis deviation was present. The cardiac silhouette was normal in a routine chest X-ray. Computed tomography of the brain showed no abnormalities. Cerebrospinal fluid was not examined.

Antidepressant therapy with imipramine (25 mg three times a day) and levopromazine (25 mg twice a day) was started on hospital admission and was continued during the whole period for reactive depression. One month later, on April 25, 1981, antitumor treatment was instituted orally with tamoxifen (20 mg/day), prednisone (50 mg/day), and cyclophosphamide (150 mg/day). In addition, weekly iv injections of 5-FU (1000 mg) and MTX (60 mg) were planned.

During the first injection of MTX the patient developed palpitations, dizziness, and nausea after 20 mg had been administered. Treatment was stopped immediately. An ECG was not done. A second attempt at MTX administration on the following day had to be interrupted again since the patient developed similar symptoms and an incomplete motor aphasia. The latter subsided over the following 3 days. On May 4, a third attempt at MTX administration was undertaken under cardiac monitor control. After 30 mg had been injected, multiple supraventricular extrasystoles were observed and the MTX was stopped. The blood pressure remained normal at 150/80. The patient reported an oppressed feeling and lost consciousness for 5–10 seconds. During this time muscle spasms were observed in the neck. The acute symptoms subsided within 3 hours. A mild motor aphasia persisted for 2 days. The ECG remained unchanged without further extrasystoles. It should be noted that the patient had received no medication prior to the second and the third MTX injections. Further attempts at MTX treatment were not made. Similar neurologic or cardiac episodes

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have not recurred during an observation period of 4 months, with otherwise unchanged treatment.

DISCUSSION

The hematologic toxicity of MTX is well-known. The drug has also been associated with stomatitis, alopecia, and hypersensitivity skin reactions. Cases of renal, hepatic, and pulmonary damage have also been reported (1). The wide variety of neurologic side effects reported in the literature have, however, only been observed following either intrathecal administration or carotid artery infusion (2). In a recent review of cardiac toxicity associated with antitumor therapy, descriptions of possible MTX-induced cardiac symptoms are mentioned (3): one patient died of congestive heart failure following bronchopneumonia due to leukopenia (4). Rosen et al (5) found a threefold increase in doxorubicin-induced ECG changes in patients previously treated with high-dose MTX and leucovorin rescue. Recently, a report of unusual MTX toxicity occurring in close temporal relationship with systemic MTX administration has appeared (6). Among others, the investigators relate three cases of loss of consciousness and one acute myocardial infarction.

In our patient, the MTX was given iv in conventional doses. On three successive occasions the MTX administration was followed by dramatic neurologic symptoms and cardiac arrhythmia. The ECG reverted to normal immediately following the third MTX injection and has remained so since. We cannot exclude an interaction between MTX and imipramine.

It would be difficult to explain the neurologic symptoms on the basis of transient cerebral ischemia. The systemic blood pressure remained normal throughout the entire period of observation before and after the MTX applications. There were no evident signs of inflammatory or metastatic lesions within the central nervous system, which could have altered the blood-brain barrier. The hypothesis of an anaphylactic reaction can be made, but we have no supportive evidence for it.

Although we cannot explain these rare reactions, their causal relation to MTX administration is highly probable. It therefore seems important to realize that such neurologic and cardiac side effects may occasionally follow iv injections of MTX in conventional doses.

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Phase II Study of Sequential Methotrexate-5-FU Therapy in Advanced Measurable Colorectal Cancer[1,2]

Brief Reports

James E. Cantrell, Jr,[3] Rene Brunet,[4] Claude Lagarde,[4] Philip S. Schein,[3] and Frederick P. Smith[3,*]

SUMMARY

Eighteen patients with previously untreated advanced colorectal cancer were treated with a sequential methotrexate-5-FU regimen. One of 16 evaluable patients achieved a partial response. The results of this and other clinical and laboratory investigations reported to date suggest that further study of different doses and schedules of sequential methotrexate-5-FU is warranted.

[Cancer Treat Rep 66:1563–1565, 1982]

A schedule-dependent cytotoxic synergism between methotrexate and 5-FU has been demonstrated by Bertino et al (1). Using a Sarcoma 180 mouse tumor model, they demonstrated a synergistic antitumor effect for the combination when the methotrexate was administered 1 hour before the 5-FU, whereas simultaneous or reverse sequence administration produced a survival effect similar to either drug given alone. They postulated that the pretreatment with methotrexate enhanced the cytotoxic action of 5-FU by increasing the amount of dTMP synthetase irreversibly bound by a ternary complex consisting of FdUMP, methotrexate, and dTMP synthetase. Cadman et al (2) confirmed the synergism in L1210 leukemia cell culture. They proposed a different mechanism of synergism based on methotrexate inhibition of purine utilization of phosphoribosyl pyrophosphate. Consequently, phosphoribosyl pyrophosphate accumulates and is diverted to use in the formation of 5-FU ribonucleotide compounds, which accumulate intracellularly, as well as in the parent drug 5-FU and FdUMP (2,3).

To date, a limited number of clinical trials using various drug doses and schedules of sequential methotrexate-directed 5-FU appear to confirm the efficacy for this approach in the treatment of breast cancer (4,5), head and neck cancer (6), and colorectal cancer (5,7,8). We report the results of a phase II trial of a sequential methotrexate-5-FU combination in previously untreated patients with advanced measurable colorectal cancer; the response rate observed was less than expected if 5-FU had been used alone.

PATIENTS AND METHODS

Eighteen consecutive patients with histologically documented metastatic colorectal carcinoma were treated with a methotrexate-5-FU sequential regimen. The median age was 57 years (range, 35–72), with a median Eastern Cooperative Oncology Group performance status of 1 (range, 0–3). The predominant sites of disease were lung (six patients) and liver (nine); other areas involved were bone, nodes, peritoneum, vagina-pelvis, and skin. None of the patients had received any prior chemotherapy. Patients had previously undergone radiation therapy to the pelvis (seven patients), bone (one), liver (one), and mediastinum (one), but none had received treatment to the site of followable disease.

The treatments consisted of 250 mg/m^2 of methotrexate followed in 1 hour by 600 mg/m^2 of 5-FU. Each drug was administered as an iv bolus. Leucovorin at a dose of 10 mg/m^2 was administered iv or orally 24 hours after the infusion of 5-FU and then orally every 6 hours for 2 days. Treatments were repeated at least every 3 weeks and usually within 2 weeks and were continued until disease progression occurred.

Response was assessed by clinical examinations,

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serial X-rays, and nuclear scans when appropriate. Patients were classified as having a complete response, partial response, stable disease, or progressive disease. Complete response was defined as the disappearance of all measurable disease for ≥ 2 months. Partial response was defined as a 50% reduction in the products of two perpendicular tumor diameters for ≥ 2 months. Progressive disease in other sites or the occurrence of new lesions negated a partial response. Stable disease was defined as either a < 50% reduction or a < 25% increase in tumor size for ≥ 2 months. Progressive disease was defined as a ≥ 25% increase in the size of measurable tumor mass or the appearance of new tumors. Survival was calculated from the day of initiation of chemotherapy. Toxicity was noted with each course and hematologic parameters were checked prior to each treatment.

RESULTS

Sixteen of the 18 patients were evaluated for response. One each was excluded due to no followable disease or lack of followup. One partial response was observed, as evidenced by a 50% reduction in vaginal-pelvic masses. This response was first noted after four treatments (10 weeks) and has persisted for > 2 months. Disease stabilization occurred in six patients and persisted for 10–30 weeks. The remaining nine patients experienced progressive disease. Median survival was 5 months (range, 2+ to 10) for the responder and for stable patients and 4 months (range, 1–5) for patients with progressive disease. Toxicity was limited to one episode of leukopenia (2300 wbcs/mm^3) and one case of mild mucositis.

DISCUSSION

This disappointing response with sequential methotrexate-5-FU chemotherapy in patients with previously untreated colorectal cancer stands in contrast to the reported response rates for colorectal and other malignancies treated similarly (table 1). There are a number of possible explanations for this apparent discrepancy.

While the results of the present clinical trial appear to be different from those reported from preceding studies, there are also conflicting experimental data relating to methotrexate-5-FU interactions. Tattersall et al (10) treated L5178Y cells in vitro with the combination and noted a less than additive effect with a schedule in which methotrexate was given 6 hours prior to 5-FU. In contrast to the results reported with L1210 tumor cells, Benz and Cadman (11) reported no synergism for the combination against a human colorectal adenocarcinoma in vitro during the first 6 hours after methotrexate exposure. Thereafter, a gradual increase in cell growth inhibition occurred, which was maximal during the last 6 hours of a 24-hour methotrexate exposure. It is therefore quite possible that if therapeutic synergism is to be achieved, it may require a very specific drug-drug interaction that is dependent on factors such as dose, schedule, and tumor specificity (12).

The 1-hour dosing interval used in this study was based on the published results of Bertino et al (1). However, their system used Sarcoma 180 mouse cells in a logarithmic growth phase and consequently may underestimate the time needed for a relatively slow-growing carcinoma to experience a comparable biochemical effect. Drapkin et al (8) and Herrmann et al (7) (table 1) achieved response rates of 33% and 60%, respectively, when they treated advanced colorectal carcinoma with a 5-FU dose comparable to that employed in the present report but with a lengthened pretreatment phase; methotrexate was administered as a 4-hour continuous infusion followed by a 3- or 7-hour delay until the 5-FU treatment. This schedule resulted in a 7–11-hour increase in methotrexate exposure time, which may have contributed to the superior results.

The dose of 5-FU employed in our treatment program may have been inadequate to exploit the full potential of this drug. The Central Oncology Group

TABLE 1.—Summary of clinical trial results

Clinical trial (ref No.)	Tumor	No. of patients	Response rate (%)
Cantrell et al (present study)	Colorectal	16	6
Tisman and Wu (5)	Colorectal	11	28
Herrmann et al (7)	Colorectal	10	60
Drapkin et al (8)	Colorectal	19	33
Gewirtz and Cadman (4)	Breast	17	53
Tisman and Wu (5)	Breast	13	23
Pitman et al (6)	Head-neck	10	100

(9) has presented results that support a high-dose loading regimen for the treatment of colorectal carcinoma with 5-FU alone. The sequential methotrexate-5-FU regimens of Tisman and Wu (5) and Herrmann et al (7) (table 1) against advanced colorectal cancer both achieved response rates superior to ours using higher doses of iv bolus 5-FU: 1500 mg/m^2 every 3–4 weeks and 900 mg/m^2 every 2–3 weeks, respectively. These results suggest that the higher doses of 5-FU are more effective.

Based on the results of our study, we conclude that the dose and schedule of sequential methotrexate-5-FU employed is not active against previously untreated advanced colorectal cancer. Future studies of this combination should be directed toward exploring larger doses of 5-FU and lengthening the time interval between administration of the drugs. Finally, regardless of which regimen modifications are employed, it appears appropriate to explore this approach in more drug-responsive tumors such as breast cancer.

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Phase II Trial of Doxorubicin Therapy for Advanced Islet Cell Carcinoma[1,2]

Brief Reports

Charles G. Moertel,* Philip T. Lavin, and Richard G. Hahn[2]

SUMMARY

Twenty evaluable patients with advanced islet cell carcinoma were treated with doxorubicin at a dosage of 60 mg/m² every 3–4 weeks. Toxicity was clinically tolerable and characteristic of doxorubicin therapy. Although all patients had previously been treated with other chemotherapy regimens, four (20%) had objective therapeutic responses persisting for 2–30½ months. Doxorubicin has definite therapeutic activity in islet cell carcinoma and is currently being evaluated in combination chemotherapy regimens.

[Cancer Treat Rep 66:1567–1569, 1982]

Islet cell carcinoma of the pancreas has a number of unique features including a usually indolent clinical course and a variety of endocrine hyperfunction syndromes that compound the effects of the carcinoma per se. In addition, this neoplasm presents unique opportunities for therapeutic exploitation, since a number of agents have been found in experimental animals to be specifically toxic to the parent cell. Of these, both tubercidin and streptozocin have been found to be active against human islet cell carcinoma, and the latter drug has an established clinical role. Streptozocin produces objective responses in only about 30% of patients however, and in general, these responses are only partial and relatively transient. This agent also has side effects that are unpleasant for most patients and intolerable for some. A clear need exists for additional active agents that may either supersede streptozocin in effectiveness and tolerability or combine with it to produce enhanced effectiveness. With the exception of scattered reports of 5-FU, there have been very few recorded studies in islet cell carcinoma with the more commonly used cytotoxic drugs, even those known to have a broad spectrum of activity. In 1976, the Mayo Clinic reported objective responses in two patients with islet cell carcinoma treated with doxorubicin (1). The present study was designed to expand that experience with the larger case-contributing potential of the Eastern Cooperative Oncology Group (ECOG).

METHODS

Patient Selection

All patients selected for study were required to have histologically proven locally unresectable or metastatic islet cell carcinoma. It was also required that each patient have measurable malignant disease to serve as an objective indicator of response to therapy. This indicator could be either tumor mass or a laboratory assay specifically related to endocrine hyperfunction, eg, serum gastrin. Malignant hepatomegaly was accepted as an indicator if the liver had been shown histologically to be metastatically involved and if a distinctly palpable liver edge extended at least 5 cm below the xiphoid process or costal margin on quiet respiration. A radioactive liver scan was accepted if it displayed a clearly demarcated perfusion defect measuring at least 5 cm in greatest diameter. Patients were excluded if they had severe malnutrition or frequent vomiting, leukopenia (< 4000 cells/mm³) or thrombocytopenia (< 150,000 cells/mm³), or any evidence of active heart disease. Therapy was delayed for at least 1 month

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after any previous radiation therapy or treatment with cytotoxic drugs.

Treatment Procedures

Doxorubicin was given by rapid injection into the tubing of an established iv infusion. Treatment was repeated at 3 weeks, at 6 weeks, and every 4 weeks thereafter. The dose for each treatment was 60 mg/m^2 unless the patient had an elevation of serum bilirubin. In the latter case, the initial dose was 40 mg/m^2, with escalation, if this dose was well-tolerated, to 60 mg/m^2 for continued therapy. Treatment was continued until there was evidence of disease progression or until a total doxorubicin dose of 500 mg/m^2 had been administered.

Therapeutic results were assessed prior to each scheduled dose of doxorubicin. An objective response was defined as reduction of a tumor by > 50% in the product of the longest perpendicular diameters. If malignant hepatomegaly was used as an indicator, the sum of measurements below the xiphoid process at each costal margin and at each costal margin at the midclavicular line had to be reduced by at least 30%. If a laboratory assay of endocrine hyperfunction was used, the abnormality had to be reduced by at least 50%.

RESULTS

A total of 25 patients were entered in the study. Three patients were declared ineligible, two because they did not have measurable parameters of malignant disease and one because therapy was not administered according to protocol method and dosage. Two patients were completely unevaluable for either toxic effects or response. Therapeutic results could therefore be evaluated in 20 patients. Their characteristics are presented in table 1. All but two patients had had disease progression after prior therapy with either streptozocin alone or streptozocin plus 5-FU. The remaining two patients had had disease progression after previous treatment with 5-FU alone. Even though all patients had had prior treatment, none were totally disabled, and 55% had an ECOG performance status of 0 or 1; ie, they were capable of working either full or part time.

Toxicity

Toxic effects were characteristic of doxorubicin therapy, with nausea or vomiting on the day of administration and the universal occurrence of alopecia. Moderate leukopenia was experienced by 47% of the 15 patients for whom adequate wbc counts were obtained to determine a nadir. In these patients, wbc count nadirs ranged from 2000 to 3900/mm^3. One patient developed nonfatal congestive heart failure after receiving a total doxorubicin dose of 400 mg/m^2.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Sex	
Male	12
Female	8
Median age in yrs (range): 54 (17–78)	
Performance status*	
0	2
1	9
2	6
3	3
Prior chemotherapy	
Streptozocin	18
5-FU	10
Semustine	2
Chlorozotocin	1
Tumor function	
None	10
Zollinger-Ellison syndrome	4
Insulinoma	3
Hypercalcemia	2
ACTH	1
Glucagon	1

*ECOG score: 0 (fully active) to 4 (totally disabled).

Therapeutic Results

Four (20%) of the 20 evaluable patients had an objective response according to the criteria above. Durations of response from the start of therapy were 2, 2, 6, and 30+ months. One of the 2-month responses was in a patient who refused to continue doxorubicin after his first dose because of nausea and vomiting. The longest response was still continuing at 30½ months, with treatment having been discontinued after the patient reached a limiting total dose of 500 mg/m^2. All responses occurred in patients with nonfunctioning tumors.

The median survival of all patients was 6 months from the start of doxorubicin therapy (range, 1½–36+ months).

DISCUSSION

This study has demonstrated that doxorubicin has definite activity in the treatment of advanced islet cell carcinoma. Its substantive activity is emphasized by the 20% response rate obtained in this group of patients, all of whom had had prior chemotherapy.

A previous ECOG study showed that the combination of 5-FU and streptozocin produced a significant improvement in response rate as well as in completeness of response compared to streptozocin alone (2). The present study indicates that, in islet cell carcinoma, doxorubicin has activity that probably at least matches that of 5-FU. Pilot studies conducted at the Mayo Clinic have shown that a nearly full dose of doxorubicin can be combined with a full dose of streptozocin. The current ECOG study involves a randomized comparison of the doxorubicin-streptozocin combination, the 5-FU-streptozocin combination, and chlorozotocin used alone.

Although it is a rare neoplasm, the islet cell carcinoma is clearly vulnerable to a chemotherapeutic attack, and substantial increase in patient survival should be an obtainable goal.

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In Vitro Evaluation of the Cardiotoxic Potential of AMSA[1]

Brief Reports

Michael C. Lowe[2,*]

In contrast to many other drug therapies, cancer chemotherapy is seldom limited by therapeutic response but more often by systemic toxicity. Because the low therapeutic indices of most oncolytic agents derive from extensions of the intended effect of these drugs (ie, the killing of cells) (1), oncologists have by necessity learned to clinically manage most of the untoward responses. However, an atypical toxicity, such as the chronic cardiotoxicity of doxorubicin or daunorubicin, is much more difficult to assess and reverse, especially when it may not become apparent until several months after treatment (2).

Recently, there have been reports of a possible cardiotoxicity caused by AMSA, an acridine currently in clinical trials. There have not yet been any reported chronic cardiotoxic reactions with AMSA, but there have been cases where acute, life-threatening cardiac arrhythmias have occurred following administration of the drug (3). The electrophysiologic effects of AMSA appear to be similar to the early reports of cardiac problems following daunorubicin administration (nonspecific electrocardiographic changes with no histologic evidence of myocardial damage) (4,5). It was not until some years later that the full spectrum and incidence of cardiotoxic reactions to doxorubicin and daunorubicin were realized (ranging from acute electrophysiologic changes to a chronic cardiomyopathy characterized by myocytolysis, vacuolization, and, ultimately, congestive heart failure) (2,6).

With the slowly developing story of anthracycline cardiotoxicity in mind, it was decided to test the effects of AMSA on isolated rat heart myocytes, cells that have recently been shown to be sensitive to several anthracyclines (7).

MATERIALS AND METHODS

Cardiac myocytes were isolated from male Sprague-Dawley rats (250–300 g) as previously described (7) and were stored in Ca^{2+}-free buffer until used (within 24 hours). Summarily, the isolation procedure required a Langendorff perfusion of the heart with Ca^{2+}-free buffer containing hyaluronidase and collagenase, followed by several digestion and purification steps. AMSA (bulk) was obtained from the Drug Development Program of the National Cancer Institute and was dissolved in a 40% (vol/vol) solution of dimethyl sulfoxide (DMSO; Fisher Scientific Co) in glass-distilled water. Test concentrations of AMSA were prepared at four times the desired final concentration and were used within 1 hour. Microscopic determinations of the maximal durations of spontaneous contractions of the isolated myocytes ($1–3 \times 10^6$ cells/ml) were made in a calcium-containing buffer (Tyrode's) on hemacytometer slides at room temperature. Control observations were made with drug-free cells in Tyrode's buffer with or without 10% DMSO (the final concentration used with drug-treated cells). Up to six determinations were made at each concentration or condition tested except when there appeared to be no effect (> 70 minutes) or when the effect was so drastic it was impossible to find any beating cells (< 1 minute). Maximal durations of beating times are reported as mean values ± SD when more than two determinations were made.

RESULTS

AMSA was soluble in 40% DMSO and distilled water up to approximately 1×10^{-3} M, although yellow crystals of AMSA appeared in the solution within 30 minutes. At 1×10^{-4} M, AMSA appeared to stay in solution for > 6 hours; occasionally, while maximal beating times were being determined, crystals of AMSA would be observed on the microscope slides, in which case the experiment was terminated.

Control cells in Tyrode's buffer beat for > 70 minutes, confirming earlier observations; the addition of

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DMSO (final concentration, 10% [vol/vol]) to the buffer did not affect control beating times. When cells were exposed to 1×10^{-3} M AMSA, it was not possible to find any contracting cells among approximately 4000 cells on each test slide. Similarly, AMSA at 8×10^{-4} M and 6×10^{-4} M concentrations prevented the cells from contracting. When less concentrated solutions of AMSA were tested, a dose-response relationship was observed, but over a very narrow concentration range (table 1). Whereas 1×10^{-4} M AMSA was indistinguishable from non-drug controls (beating times > 70 minutes), 4×10^{-4} M AMSA stopped all contractions within 5 minutes. Contracting cells simply ceased to beat, arresting in various states or configurations (some cells were elongated; others were obviously supercontracted).

DISCUSSION

AMSA has been found to be toxic to spontaneously contracting isolated rat heart myocytes. Compared to doxorubicin or daunorubicin in the same test system, AMSA is approximately one tenth as potent, having an ID50 (that concentration at which the last contracting cell would cease to beat at one half the control maximal beating time) of 1.7×10^{-4} M versus 1.5×10^{-5} M for doxorubicin or daunorubicin (which are indistinguishable in this system).

Although the mechanism(s) by which anthracyclines affect the isolated heart cells used in this study is unknown, the relative specificity of the effect has been intriguing. Other anticancer drugs (methotrexate, mitomycin, 5-FU, etc) and agents known to be extremely toxic to cells in tissue culture (ie, dactinomycin) were nontoxic when tested (7).

Cardiovascular pharmacology studies of AMSA in dogs (8) predicted the acute electrophysiologic effects of the drug in man, and hemodynamic responses were only seen at high doses. In an attempt to determine the chronic cardiomyopathic potential of AMSA, the drug was tested in rabbits with weekly injections over 12 or 26 weeks (9). At the dose (determined by an acute LD50 determination) studied, AMSA was found to be marginally cardiotoxic (two of six high-dose animals had mild histologic changes in the heart). It is possible that the dose of AMSA given in the study (one tenth of the LD50 weekly) was too low and was not sufficient to show a chronic doxorubicin-type of cardiotoxicity because of the overriding acute (electrophysiologic) toxicity of AMSA.

Both dog and rabbit studies used the clinical formulation of AMSA, which contains *N*,*N*-dimethylacetamide (DMA) as a solvating agent. Von Hoff et al (3) have raised the possibility that DMA might contribute to the electrophysiologic effects observed with AMSA. Although that question was not addressed in this study, DMA was obviously not responsible for the observed effects of AMSA on heart cells, since the clinical formulation was not used.

The similarity of AMSA to the anthracyclines in the isolated myocyte system should perhaps inject a note of caution into the clinical use of AMSA. Until it is known by what mechanisms anthracyclines act acutely (electrophysiologically) or chronically (cardiomyopathically), it must be considered that AMSA might be acting in a similar way. The fact that early reports of anthracycline cardiotoxicity centered about acute electrophysiologic effects (that were discerned long before the later developing congestive heart failure syndrome) should alert oncologists to watch for cardiac performance changes in patients receiving AMSA.

TABLE 1.—Dose-response effects of AMSA on maximal durations of beating times of isolated adult rat heart myocytes

Group (No. of determinations)	Maximal duration of beating, in mins (mean ± SD)
Controls	
Tyrode's buffer (2)	> 70
10% DMSO:90% Tyrode's buffer (2)	> 70
AMSA in 10% DMSO:90% Tyrode's buffer	
1×10^{-4} M (6)	71.6 ± 12.3
2×10^{-4} M (6)	32.0 ± 9.7
4×10^{-4} M (6)	4.5 ± 1.4
6×10^{-4} M (2)	< 1
8×10^{-4} M (2)	< 1
1×10^{-3} M (2)	< 1

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Effects of Interferon on C1300 Mouse Neuroblastoma[1,2]

Brief Reports

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SUMMARY

The effects of murine interferon (MuIFN) with and without cyclophosphamide (CY) were evaluated in C1300 neuroblastoma-bearing mice. Mean survival times were prolonged by administration of 25,000 IU of MuIFN three times per week by ip injection. The combination of MuIFN and CY was more effective in the treatment of mouse neuroblastoma than either agent alone. Our results suggested that MuIFN is synergistic in combination with moderate amounts of CY in mouse neuroblastoma cells and may be given for the treatment of neuroblastoma.

[Cancer Treat Rep 66:1575–1577, 1982]

Most cases of neuroblastoma have an unfavorable prognosis in spite of intensive treatment regimens. New regimens are necessary to improve the prognosis for patients with this tumor.

In 1974, Strander et al (1) suggested that human leukocyte interferon had some beneficial effects on patients with osteosarcoma. Thereafter, interferon was experimentally administered to patients with various malignant diseases (2,3). In animal experiments, Gresser et al (4) showed that murine interferon (MuIFN) was effective on spontaneous lymphomas in AKR mice and that the combination of MuIFN and cyclophosphamide (CY) was more effective in tumor therapy than either MuIFN or CY alone.

In this paper, we report a study of the antitumor effects of MuIFN and the combination of MuIFN and CY on C1300 neuroblastoma-bearing A/J mice.

MATERIALS AND METHODS

Mice and tumor.—Inbred A/J female mice, 6–12 weeks old, were used in this study. A syngeneic C1300 neuroblastoma tumor was maintained by serial transfers in vivo. For this study, A/J mice were inoculated with 1×10^6 C1300 neuroblastoma cells sc in the interscapular area.

Murine interferon.—MuIFN was prepared at our laboratory for this study. It was induced on a monolayer of L_{929} cells infected with Newcastle disease virus (Miyadera strain). The specific activity of the L cell was 1.4×10^6 IU/mg of protein (5).

Experiment.—Four groups were prepared for this study (fig 1). In group 1, the control group, each mouse was treated with a 0.25-ml saline solution three times each week. Group 2 was given 20 mg/kg of CY. Group 3 was treated with MuIFN at a dose of 25,000 IU/mouse three times each week. Group 4 was given a combination of MuIFN and CY at the same doses as groups 2 and 3. Saline, MuIFN, and CY were administered by ip injection. These treatments were started on Day 8 after inoculation of tumor cells, when a palpable mass could be detected, and were continued until death of the animal. This experiment terminated on Day 75 after inoculation of tumor cells. The survival times of the two living mice in group 4 were estimated to be > 75 days. The significant differences in mean survival times for each group were tested by Student's t test.

RESULTS

The effects of MuIFN and/or CY administration on tumor-bearing mice were evaluated by survival time and incidence of tumor regression in each group compared with those of the control group. The re-

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Group	Treatments	No. of Mice	Survival days (mean SD)*	Survivors on day 70
1	Saline,0.25mlx3/W	8	24.1± 1.9	0/8
2	CY, 20mg/kgxl+Saline	7	30.3± 2.9	0/7
3	L-cell IFN,25,000IUx3/W	7	33.0± 3.2	0/7
4	L-cell IFN+CY	8	>50.7± 5.4	2/8

* Significance of difference of mean survival days; (1)vs(3):p<0.05, (1)vs(4):p<0.001, (2)vs(4):p<0.01 and (3)vs(4):p<0.02.

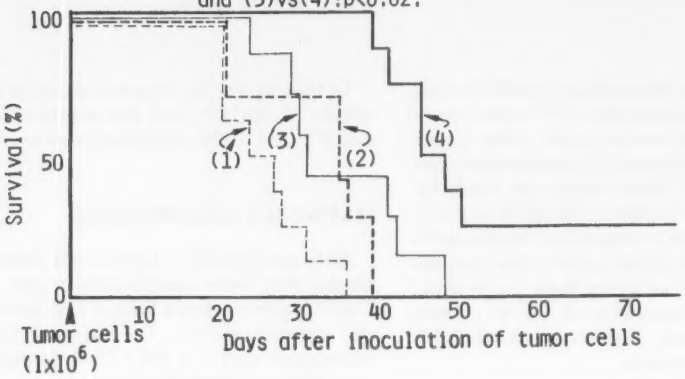

FIGURE 1.—Effects of MuIFN and CY on survival times of C1300 neuroblastoma-bearing mice.

sults are shown in figure 1. MuIFN was effective in prolongation of survival of the tumor-bearing mice ($P < 0.05$). Although 20 mg/kg of CY was considered to be effective in prolonging survival, there was not enough statistical evidence ($P > 0.05$). Group 4, receiving combination therapy with MuIFN and CY, showed a more prolonged survival than either of the groups receiving MuIFN or CY alone. Total regression of the tumor mass was noted in two of the eight mice of group 4; those two animals survived for > 75 days after inoculation of tumor cells.

DISCUSSION

This study was the first trial of MuIFN administration to C1300 neuroblastoma-bearing mice. In this study, administration of MuIFN and/or CY was started on Day 8 after inoculation of 1×10^6 tumor cells, when a palpable mass could be detected. A preliminary study was carried out using MuIFN of different origins and different doses of MuIFN or CY. Two MuIFNs were prepared for this study: L-cell MuIFN and brain MuIFN (extracted from the brains of ICR mice inoculated with Japanese encephalitis virus [JaGAr 01]). Survival times (mean ± SE) of the five tumor-bearing mice treated with 25,000 IU of brain MuIFN and L-cell MuIFN (28.2 ± 4.5 and 29.4 ± 3.0 days, respectively) were longer than the mean survival time of five mice treated with 2500 IU of brain MuIFN (23.3 ± 4.4 days). However, the difference between survival times after doses of 25,000 and 2500 IU of MuIFN was not significant. There was also no statistical difference in the mean survival period with MuIFNs from different origins.

In the preliminary studies of chemotherapy for neuroblastoma, a high dose of CY (230 mg/kg) was reported to be the most effective dose in prolonging the survival of C1300 neuroblastoma-bearing A/J mice (6). Since many mice died from side effects of the high dose of CY, it was reduced to 20 and 40 mg/kg for this study. There was no significant difference in mean survival between the group receiving 20 mg/kg of CY and the control group; however, seven tumor-bearing mice injected with 40 mg/kg of CY had prolonged mean survival times ($P < 0.01$). As a result, to eliminate the harmful side effects of CY and to evaluate the effects of combination therapy with MuIFN and CY, the CY dose was reduced to 20 mg/kg.

Tumor masses of two group 4 mice totally regressed after combination therapy, and both mice were alive at the termination of this study.

These results showed that combination therapy with MuIFN and CY may be a favorable new regimen, and the mechanism of regression of C1300 neuroblastoma is now being studied.

There have been two studies on the effects of combination therapy with MuIFN and a cytotoxic drug for leukemia and lymphoma. Chirigos and Pearson (7) reported the striking efficacy of MuIFN and carmustine (BCNU) for treatment of murine leukemia. Gresser et al (4) reported the efficacy of MuIFN and CY for treatment of spontaneous AKR lymphoma. Our combination MuIFN-CY therapy also showed success in the treatment of C1300 neuroblastoma. Doses of MuIFN and CY were lower than those in the previous report (4); however, our combination showed more inhibitory effects on tumor growth than either MuIFN or CY alone. We concluded that the prolonged survival of tumor-bearing mice resulted from synergistic effects of MuIFN and CY on tumor growth.

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Parenteral Formulation of Hexamethylmelamine Potentially Suitable for Use in Man[1,2]

Brief Reports

Matthew M. Ames* and John S. Kovach[3]

SUMMARY

We have developed a preparation of hexamethylmelamine (HMM), dissolved in a fat emulsion Intralipid 20%, that is potentially satisfactory for parenteral administration to man. Solid HMM can be dissolved in Intralipid 20% up to 5 mg/ml, with extensive agitation. Solubilization of HMM is facilitated first by dissolving the drug in ethanol or in dimethylacetamide and then by adding the solution to Intralipid 20%. The disposition of HMM in rabbits after iv administration of HMM dissolved in Intralipid 20% was similar to the disposition of the drug following iv administration of HMM dissolved in 0.1 N HCl.

[Cancer Treat Rep 66:1579–1581, 1982]

Hexamethylmelamine (HMM) is an antitumor agent with activity against several human malignancies (1). Because of the lack of a suitable parenteral preparation, HMM has been administered orally to man. Oral administration of HMM to rabbits (2) and to man (3) results in low and variable concentrations of drug in plasma. We have demonstrated that the low concentration of HMM in plasma after oral administration to rabbits results from extensive metabolism by the liver (first pass effect) rather than from poor absorption from the gut (2). There are no quantitative data concerning the absorption of HMM in man.

Several parenteral preparations of HMM have been studied. These include the free base dissolved in 0.1 N HCl and the hydrochloride and gentisate salts dissolved in water. The acid vehicle produced moderate local irritation and venous thrombosis in large animals (4). The gentisate salt of HMM produced severe local irritation in animals (5) and was unstable at physiologic pH (6). The administration of HMM hydrochloride iv to beagle dogs was reported recently to be well-tolerated (7). However, in a limited clinical trial conducted in 1962, the hydrochloride salt caused severe local irritation and phlebitis in man (B. Morrision, III, National Cancer Institute [NCI]: memo to operating committee).

The NCI recently developed pentamethylmelamine (PMM), a water-soluble analog of HMM, for clinical testing by parenteral administration (5). HMM and PMM have been reported to have comparable activity in vivo against transplantable tumors in rodents (5). PMM administered iv to man in phase I trials did not produce significant cytotoxicity at doses causing intolerable gastrointestinal and central nervous system toxicity (8–12). To our knowledge, no phase II trials of PMM are planned. Because we believe that the antitumor activity of parenterally administered HMM should be evaluated in man, we have been attempting to develop a formulation of HMM suitable for iv administration. We report that HMM in Intralipid 20% appears suitable for testing as a parenteral formulation of HMM in man.

MATERIALS AND METHODS

Intralipid 20%, a fat emulsion consisting of 20% soy bean oil, 1.2% egg yolk phospholipids, and 2.25% glycerine in water, was purchased from Cutter Laboratories, Berkeley, CA. HMM was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI, Bethesda, MD. Filters (0.45 μ) were purchased from Millipore Filter Company, Milford, MA.

The hydrochloride salt of HMM was prepared by dissolving HMM in ether and bubbling gaseous HCl through the ether solution followed by filtration and recrystallization of the precipitated salt. HMM and its hydrochloride salt were dissolved in aqueous solution or in Intralipid 20% by shaking for 2 hours

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in screw-top centrifuge tubes at room temperature. HMM was also dissolved in ethanol at 5, 10, and 20 mg/ml and in dimethylacetamide (DMA) at 20, 40, and 50 mg/ml. The HMM-ethanol or HMM-DMA solutions were added to a stirred solution of Intralipid 20%. The concentration of HMM was measured by a nitrogen-phosphorus gas chromatographic assay following extraction of alkalinized aliquots of the aqueous and lipid solutions with toluene as previously reported (13).

New Zealand white rabbits (2.5–3.1 kg) were injected iv in an ear vein with HMM dissolved in a minimal volume of 0.1 N HCl, HMM dissolved in Intralipid 20%, or HMM dissolved in ethanol or DMA and then added to Intralipid 20%. Samples of blood were collected in heparin tubes from the uninjected ear.

RESULTS

The maximum amount of HMM which could be dissolved in water was 0.20 mg/ml. The hydrochloride salt of HMM dissolved readily in water or 0.9% sodium chloride up to 20 mg/ml. The pH of solutions of HMM hydrochloride in water or 0.9% sodium chloride ranged from 3 to 2 at concentrations from 1 to 20 mg/ml, respectively. Measurement of HMM in toluene extracts of Intralipid 20% after addition of known amounts of solid HMM to the lipid preparation showed almost complete dissolution (allowing for extraction efficiency) at a theoretic concentration of 5 mg/ml (89.5% ± 6.6%). HMM was not completely dissolved in Intralipid 20% at a theoretic concentration of 10 mg/ml (58.4% ± 4.9%).

To facilitate complete and reproducible dissolution of HMM in Intralipid 20%, we dissolved HMM in ethanol (14) or in DMA. HMM dissolved in ethanol with warming to 37°C up to 20 mg/ml. At 10 mg/ml in ethanol, HMM remained in solution at room temperature for at least 2 hours. At 20 mg/ml in ethanol, HMM remained in solution at room temperature for at least 10 minutes. HMM dissolved in DMA at room temperature up to 50 mg/ml. Addition of HMM in ethanol at 20 mg/ml to Intralipid 20% achieved a maximal concentration of 2 mg/ml of HMM (10% ethanol) without disturbance of the homogeneity of the emulsion. Addition of HMM in DMA at 50 mg/ml to Intralipid 20% achieved a maximal concentration of 2 mg/ml of HMM (4% DMA) without disturbance of the emulsion. Analysis of toluene extracts of HMM in the ethanol and Intralipid 20% solution and in the DMA and Intralipid 20% solution showed quantitative recovery of drug after filtration through a 0.45-μ filter. Recovery of HMM from the DMA preparation was 95% ± 1.0% and from the ethanol preparation, 93.0% ± 0.6%. Samples stored at room temperature for 24 hours were unchanged in their content of HMM.

We studied the disposition of HMM in rabbits after iv administration of the lipid preparations. Peak plasma concentrations and rates of elimination of HMM from plasma were similar for the three preparations, and virtually identical to the characteristics of the drug when given dissolved in dilute HCl (3). Irritation of the ear of rabbits receiving the drug was always noted after the injection of acidic formulations of HMM, but not after injection of any of the three lipid preparations.

DISCUSSION

A preparation of HMM dissolved in Intralipid 20% appears suitable for iv administration to man. Fat emulsions and related systems have been used previously for parenteral administration of lipid-soluble antitumor agents including lomustine (CCNU), carmustine (BCNU), semustine (methyl-CCNU), bleomycin, and 5-FU (14–16).

Rapid and complete dissolution of HMM in the lipid was facilitated by first dissolving the drug in ethanol or in DMA. Either of these formulations appears suitable for iv administration to man. Administration of 0.5 g of HMM would necessitate administering 25 ml of alcohol (250 ml of 10% ethanol-lipid mixture containing 2 mg/ml of HMM) or 10 ml of DMA (250 ml of 4% DMA-lipid mixture containing 2 mg/ml of HMM), amounts of these two solvents which have been administered safely to man (17,18). Concentrations > 2 mg/ml of HMM in Intralipid 20% can be achieved by dissolving solid HMM in the lipid directly. Because the opacity of the lipid prevents visual assessment of the completeness of dissolution, it will probably be necessary to first dissolve HMM in one of the two solvents and then add the completely dissolved drug to Intralipid 20%.

We believe HMM administered iv should be evaluated for antitumor activity in man. HMM, but not its water-soluble metabolite PMM (8–12), has been demonstrated to be active against several human cancers. We have demonstrated that NADPH-dependent covalent binding of HMM in vitro is five times greater than NADPH-dependent covalent binding of PMM to microsomal macromolecules (19,20), a difference which may be related to antitumor activity of HMM compared to PMM. We have also documented in rabbits that the large first pass effect limits markedly the plasma concentration of HMM achievable after oral administration (2). For these reasons we believe that parenteral HMM may produce pharmacologic effects at much lower total doses than are customarily given orally. This parenteral formulation of HMM may also be of use for

parenteral administration of HMM via routes other than iv, as has been tested in the treatment of advanced ovarian carcinoma.

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Phase II Trial of Prednimustine in Children With Recurrent Cancer: A Children's Cancer Study Group Report[1,2]

Clinical Trials Summaries

Paul S. Gaynon,* Edward S. Baum, William Krivit, and Denman Hammond[2]

Prednimustine is an ester of chlorambucil and prednisolone. Early trials in adults showed activity in breast cancer, chronic lymphocytic leukemia, acute myeloblastic leukemia (AML), non-Hodgkin's lymphoma (NHL), ovarian carcinoma, and prostatic carcinoma. The Children's Cancer Study Group designed a phase II trial, CCG-083, largely restricted to acute leukemia and NHL, where adult data suggest activity.

Of 141 patients entered, 99 were evaluable. The patients, all previously treated, received one of four regimens: *regimen D*—40 mg/m²/day for 28 days minimum; *regimen E*—40 mg/m²/day for 14 days with escalation to 60 mg/m²/day for 14 days minimum; *regimen I/200*—200 mg/m²/day for 5 days every 14 days; and *regimen I/300*—300 mg/m²/day for 5 days every 14 days. Seventy patients had acute lymphoblastic leukemia (ALL), 21 had AML, four had NHL, two had neuroblastoma, one had chronic myelogenous leukemia (CML) in blast cell crisis, and one had hepatocellular carcinoma (table 1).

In ALL, 12 objective responses were seen. Ten of these were minimal responses; eight patients had transient clearance of peripheral blast cells and two had markedly decreased organomegaly, with no improvement in marrow status. Two patients had PR, with reductions in the marrow from > 25% blast cells to between 5% and 25% blast cells. Neither response lasted 4 weeks. There were no CR seen in the marrow (< 5% blast cells). Both 60 mg/m²/day and 200 mg/m² on the intermittent schedule were adequate to suppress counts independently of clearing blast cells from the bone marrow.

The one patient with CML achieved an M2 marrow that lasted 4 months with regimen E. The one patient with hepatoma obtained a > 50% reduction in his primary tumor, as shown by computerized tomographic scan, that lasted 6 months with regimen I/200. The PR was accompanied by an increased sense of well-being.

Toxic effects included nausea and vomiting, hematemesis, abdominal pain, elevated transaminases with or without elevated bilirubin, and rashes. Of note, ten patients had seizures: eight grand mal and two myoclonic. In four patients the seizures were associated with intracranial hemorrhage; in six pa-

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tients no etiology was determined. Two patients died with interstitial pneumonia: one had received only 2 days of therapy; the other had received 120 mg/m^2/day for > 1 month.

TABLE 1.—Patient characteristics: response and toxicity

Characteristic	No. of patients			
Entered			141	
Evaluable for response			99	
Age in yrs				
Median: 9				
Range: 10 mos–23 yrs				
Sex				
Male			91	
Female			50	
Responses/trials for ALL	*MR*	*PR*	*CR*	*Trials**
Regimen D	3	1	0	19
Regimen E	5	1	0	27
Regimen I/200	1	0	0	13
Regimen I/300	1	0	0	11
Toxic effect				
Nausea and vomiting			17	
Abdominal pain			9	
Elevated transaminases			8	
Diarrhea			5	
Hematemesis			4	
Hypertension			3	
Rash			2	
Interstitial pneumonia			2	
Seizures			10	
Regimens D and E			1	
Regimens I/200 and I/300			9	

*MR = minimal responses; PR = partial responses; and CR = complete responses.

Both seizures (1,2) and interstitial pneumonia (3,4) have been reported with chlorambucil administration. The abdominal pain and hematemesis may be attributed to prednisolone side effects.

The uniqueness of prednimustine is confounded by the observation of Kirdani et al (5) that the drug is broken down quantitatively in the gut and no intact ester may be detected in the blood at any time. The lesser myelosuppression is due to lesser, if unpredictable, absorption. The unpredictability of absorption may be the cause of the seizures observed primarily in children receiving regimens I/200 and I/300.

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Phase II Trial of Combination Methyl-GAG and Vinblastine in the Treatment of Metastatic Renal Adenocarcinoma[1,2]

Clinical Trials Summaries

Robert F. Todd, III, Marc B. Garnick,* and George P. Canellos[3,4]

Metastatic renal adenocarcinoma remains largely resistant to effective systemic therapy including chemotherapy. In a phase I–II trial, we recently reported that methyl-GAG administered weekly produced an objective response rate of 16% (one complete and three partial responses) in a series of 25 patients with this disease (1). A similar experience was reported by Knight et al (2). Myelosuppression was an infrequent drug-related side effect in these studies. Lack of this toxic reaction prompted a followup phase II trial consisting of methyl-GAG plus another marginally active agent, vinblastine (3,4), whose major dose-limiting side effect is myelosuppression. In this report, we describe our clinical experience using combination methyl-GAG and vinblastine in the treatment of metastatic renal adenocarcinoma.

Table 1 shows the pretreatment characteristics of 15 evaluable patients with measurable metastatic renal adenocarcinoma who were entered in the protocol. Two of the four patients who had received prior chemotherapy had been started on single-agent methyl-GAG for 3 and 4 weeks immediately before entrance in the combination protocol (pending approval of the combination study). No patients had received prior vinblastine. The treatment plan consisted of vinblastine administered by bolus iv infusion at a dose of 5 mg/m^2 on Day 1 of a 21-day treatment cycle; methyl-GAG was given by bolus iv infusion (over 45–60 mins) at a dose of 500 mg/m^2 on Days 2 and 8. At the start of this study, three patients were treated with vinblastine at a dose of 5 mg/m^2 on Days 1 and 2, but this resulted in unacceptable myelosuppression in two individuals (wbc count < 500/mm^3), prompting the subsequent administration of vinblastine on Day 1 only. The appearance of significant toxic reactions (table 1) resulted in discontinuation of both drugs until toxicity abated, followed by dose reduction of 10% (if wbc count was > 1000 but < 2000/mm^3 and platelet count

TABLE 1.—Patient characteristics, response, and toxic reactions in phase II trial of methyl-GAG and vinblastine in the treatment of advanced renal adenocarcinoma

	No. of patients
Patients evaluable (male/female)	15 (13/2)
Median age in yrs (range): 59 (16–76)	
Median performance status (range): 1 (0–2.5)	
Prior treatment	
Nephrectomy	13
Chemotherapy (doxorubicin, vincristine, dactinomycin, methyl-GAG)	4
Hormone therapy (Megace, Provera)	4
Radiation therapy (localized)	10
Sites of measurable disease	
Lung	13
Soft tissue mass	6
Bone	2
Liver	1
Skin	1
Median No. of courses of methyl-GAG + vinblastine (range): 3 (1–11)	
Partial or complete responses	0
Toxic reactions	
Nausea/vomiting	2
Weight loss (> 10% baseline)	5
Neuralgia/myalgia	6
Mucositis	2
Balanitis	1
Myelosuppression (total)	10
Leukopenia (< 3000 wbcs/mm^3)	7
Thrombocytopenia (< 100,000 platelets/mm^3)	1
Anemia (transfusions required)	6

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was > 50,000 but < 100,000/mm^3) or 50% (if wbc count was < 1000/mm^3, platelet count was < 50,000/mm^3, and severe nausea and vomiting as well as severe neuromuscular symptoms existed). All patients receiving a minimum of one complete 21-day cycle of therapy were evaluable for response and toxicity. Pertinent studies documenting extent of disease were repeated at 3–6-week intervals.

Using the accepted criteria for response (1), there were no documented complete or partial responses in this group of 15 evaluable patients (table 1). Toxic effects attributed to therapy are indicated in table 1; myelosuppression was the major toxic reaction, affecting 67% of the patients treated.

Based on this experience with these 15 evaluable patients, combination chemotherapy with methyl-GAG and vinblastine as administered according to this dose and schedule regimen does not improve on the minimal efficacy of these when given as single agents (4,5) in the treatment of metastatic renal adenocarcinoma.

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Phase II Evaluation of Maytansine in Refractory Non-Hodgkin's Lymphoma: A Southwest Oncology Group Study[1,2]

Clinical Trials Summaries

Voravit Ratanatharathorn,[3] Nazli Gad-el-Mawla,[4] Henry E. Wilson,[5] John D. Bonnet,[6] Saul E. Rivkin,[7] and Robert Mass[8,*]

Maytansine is a naturally occurring ansa macrolide originally isolated by Kupchan et al (1) from the East African shrub *Maytenus ovatus* and later from the wood and bark of *Maytenus buchananii*. The mechanism of action involves inhibition of mitotic spindle formation (2). In the experimental tumor screen, it was found to have significant activity against P388 leukemia, B16 melanoma, and Lewis lung carcinoma. A phase I study conducted at Wayne State University using a weekly schedule demonstrated tolerable toxicity, and maytansine was adopted for group-wide clinical trials in refractory non-Hodgkin's lymphoma (3).

Nineteen patients were entered in the study. Maytansine was administered by iv bolus at a dose level of 0.75 mg/m^2/week in 16 patients. For the three poor-risk patients, defined as those with abnormal liver function (values of transaminase, alkaline phosphatase, or bilirubin more than three times normal) or previous exposure to vinca alkaloid with residual disabling neuropathy, the dose was decreased to 0.5 mg/m^2/week. Subsequent doses were escalated by an increment of 0.25 mg/m^2 if no toxicity was encountered after 4 weeks of treatment. All patients with non-Hodgkin's lymphoma had been previously treated with a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone; of these, 13 had also been treated with various combinations that contained several other drugs including methotrexate, cytarabine, bleomycin, a nitrosourea, and DTIC.

Criteria for response were defined as follows: *complete response*—the disappearance of all clinical evidence of active tumor, and *partial response*—a $\geq$ 50% reduction in the sum of the products of the perpendicular diameters of all measured lesions. Response must have been maintained for at least 4 weeks to have been considered significant.

Two patients were excluded from evaluation due to early death from rapidly progressive disease. An average of five weekly injections were administered (range, two to 12 injections). Of the 17 evaluable patients, three received a starting dose of 0.5 mg/m^2, and 14 received 0.75 mg/m^2. Subsequent doses were escalated from 0.75 to 1.0 mg/m^2 in four patients and from 0.5 to 0.75 mg/m^2 in one patient. Only one patient had dose de-escalation because of thrombocytopenia after four weekly treatments of 0.75 mg/m^2. The median wbc and platelet count nadirs were 5.4 $\times$ 10^3/mm^3 (range, 1.4–21.2 $\times$ 10^3) and 160 $\times$ 10^3/mm^3 (range, 19–323 $\times$ 10^3), respectively. Two patients developed severe myelosuppression; both received 0.75 mg/m^2 for four and eight doses. Other toxic effects included weakness and paresthesia in three patients (who received four, five, and 12 weekly doses); the latter patient also developed severe diarrhea. One additional patient had diarrhea after receiving only two doses. All three patients who developed neuropathy had received four or more doses; two of these were started at 0.5 mg/m^2 because of pre-existing hepatic dysfunction.

However, neuropathy was not seen in four patients who received subsequent dose(s) of 1.0 mg/m^2 after four weekly injections of 0.75 mg/m^2, although none had received more than two escalated doses. Cumulative neurologic toxicity was not apparent but seemed to be related to pretreatment liver function abnormality. Partial remission was seen in one patient, lasting for only 4 weeks.

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We therefore conclude that maytansine has no significant antitumor activity in heavily pretreated patients with non-Hodgkin's lymphoma.

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Phase II Trial of AMSA in Previously Treated Patients With Stage III and IV Ovarian Cancer[1,2]

Clinical Trials Summaries

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AMSA is an acridine derivative whose cytotoxic effects are believed to be mediated by intercalation with, and external binding to, DNA (1). The drug was originally selected for clinical trials because it is an active antitumor agent for a diverse group of murine tumors including B16 melanoma, L1210 and P388 leukemias, and CD breast and Lewis lung carcinomas (2). Phase I clinical trials conducted at several centers demonstrated activity of AMSA in a variety of human neoplasms, including acute leukemia, breast cancer, and ovarian cancer (3–5). We have conducted a phase II investigation of AMSA in patients with International Federation of Gynecology and Obstetrics stage III and IV ovarian cancer who had previously been treated with chemotherapy.

PATIENTS AND METHODS

Twenty patients with histologically confirmed metastatic ovarian cancer were treated with AMSA. Entry in the study began in February 1979 and was concluded in March 1980. In all cases, pathologic material was reviewed by the Department of Pathology, Memorial Sloan-Kettering Cancer Center. Complete initial medical histories and physical examinations were recorded on standard evaluation forms. Automated blood and platelet counts were obtained weekly; performance statuses (Karnofsky scale) and screening biochemical profiles (including liver functions tests) were evaluated at least once every 3 weeks; and chest X-rays and radionuclide scans were obtained every 3–6 weeks, where indicated, to evaluate response.

Criteria for patient selection included acceptable hematologic status (leukocyte and platelet counts $\geq$ 4500 and 175,000/mm^3, respectively), the presence of bidimensionally measurable lesions, performance status $\geq$ 50%, and no evidence of significant renal or hepatic dysfunction (serum creatinine $<$ 2.0 mg/100 ml; serum bilirubin $<$ 1.5 mg/100 ml).

The median age for the 20 patients treated was 62 years (range, 47–75) and the median performance status was 80% (range, 50%–100%). Fifteen patients were previously treated with chemotherapy alone, and five had previously received chemotherapy and radiation therapy. All prior treatment had been discontinued for at least 3 weeks prior to the initiation of treatment with AMSA.

Measurable lesions consisted of abdominal and pelvic masses (14 patients), subcutaneous metastases (five), metastatic nodal disease (four), and hepatic (four) and pulmonary (three) metastases.

AMSA was administered as an iv infusion over a 30-minute period once every 3 weeks. The initial dose was 120 mg/m^2 for patients with good marrow reserve, while patients who had received extensive pelvic irradiation or multiple courses of chemotherapy with substantial prior bone marrow toxicity were begun at a dose of 90 mg/m^2. The dose was increased from 90 to 120 mg/m^2 if the leukocyte and platelet count nadirs were $\geq$ 3000 and $\geq$ 100,000/mm^3, respectively. The dose was reduced from 120 to 90 mg/m^2 if the leukocyte and platelet count nadirs were $<$ 3000 and $<$ 100,000/mm^3, respectively.

An adequate trial was defined as two doses of AMSA with myelosuppression or one dose with myelosuppression and rapid disease progression. Response criteria were as follows: *partial remission*—a $\geq$ 50% reduction in the sum of the products of the largest diameters of all measurable lesions for $\geq$ 1 month; *minor remission*—a $\geq$ 25% but $\leq$ 50% decrease in measurable lesions for $\geq$ 1 month or a $\geq$ 50% decrease for $<$ 1 month; *stable disease*—a $<$ 25% increase or decrease in tumor size; and *disease progression*—a $\geq$ 25% increase in the sum of the products of the largest diameters of all measurable lesions.

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TABLE 1.—Hematologic toxicity of AMSA

Dose (mg/m^2)	No. of patients	No. of courses	Leukocyte count nadir (cells × $10^3/mm^3$) Median (range)	Leukocyte count nadir (cells × $10^3/mm^3$) Day	Platelet count nadir (cells × $10^3/mm^3$) Median (range)	Platelet count nadir (cells × $10^3/mm^3$) Day
90	13	20*	2.7 (0.9–7.3)	7	156 (70–346)	8
120	13	20†	1.9 (0.3–3.2)	14	125 (20–218)	14

*Additional 2 courses not evaluable due to lack of interim counts.
†Additional 3 courses not evaluable due to lack of interim counts.

RESULTS

All patients had an adequate trial. Thirteen patients received 90 mg/m^2, and 13 received 120 mg/m^2. Toxic effects included nausea and vomiting, which were mild and occurred in one half of the patients treated, and myelosuppression, which occurred at both dose levels (table 1). There was no evidence of renal or hepatic injury secondary to treatment with AMSA as reflected in measurements of serum BUN, creatinine, bilirubin, alkaline phosphatase, and SGOT. None of the patients responded to treatment with AMSA. As of July 1980, the median survival time from initiation of protocol to followup or death was 7 months (range, 1–17+).

CONCLUSIONS

This trial of AMSA in 20 patients with advanced ovarian cancer has produced significant myelosuppression without beneficial therapeutic effect. The results suggest that AMSA is an inactive agent in ovarian cancer.

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Phase II Evaluation of Gallium Nitrate in Breast Cancer: A Southwest Oncology Group Study[1,2]

Clinical Trials Summaries

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Gallium nitrate is a II_A metal salt which has been shown to concentrate in breast cancer tissue and hormonally stimulated normal breast tissue (1,2). We embarked on a phase II study of gallium nitrate in previously treated patients with breast cancer following phase I studies which indicated that 700 mg/m² every 2 weeks was a safe dose [(3); M. K. Samson, unpublished data].

MATERIALS, METHODS, AND RESULTS

All 26 heavily pretreated patients with metastatic breast cancer who were entered in the study were eligible. Eligibility requirements included measurable disease, life expectancy ≥ 6 weeks, BUN ≤ 20 mg/100 ml, creatinine ≤ 1.2 mg/100 ml, creatinine clearance > 60 ml/minute, two functioning nonobstructed kidneys, bilirubin < 2 mg/100 ml, no evidence of hyperuricemia or hypercalcemia, and no concomitant chemotherapy or radiotherapy. Patients were given 700 mg/m² of gallium nitrate in 250 ml of normal saline over 30 minutes following prehydration with 1500 ml of 5% dextrose in one-half normal saline every 2 weeks. An adequate trial was considered to consist of two courses of therapy.

Three of the 26 eligible patients received less than two courses of therapy (early death) and were considered to be partially evaluable. Among the 23 patients considered to be fully evaluable, there were no responses. The median number of courses of gallium nitrate received was three (range, two to nine courses), and the median performance status was 2 (symptomatic but ambulatory). Of the patients who might be expected to respond, ie, two with positive gallium scan, two who were estrogen-receptor positive, and one who was premenopausal, all developed progressive disease.

Toxic reactions among all 26 partially and fully evaluable patients were minimal, with five patients developing mild nausea and six developing minimal renal dysfunction (BUN > 20–< 40 mg/100 ml and/or creatinine > 1.2–< 2 mg/100 ml). One patient developed severe renal dysfunction preterminally due to tumor. Median survival for all patients was 26 weeks.

DISCUSSION

Despite reports of uptake of gallium in patients with breast cancer (1), particularly hormonally stimulated tissue (2), gallium nitrate did not show any significant activity in this heavily pretreated patient population.

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Case Report: Acute Leukemia After Semustine (Methyl-CCNU) Therapy[1]

Second malignancies have been the subject of many investigations involving patients previously treated for malignant lymphoma and myeloma. Radiation therapy and alkylating agents are among the major therapeutic regimens implicated. There have been several recent reports of acute leukemia following the administration of the nitrosoureas. The following is a case report of a patient who developed acute nonlymphocytic leukemia after treatment with semustine (methyl-CCNU) and 5-FU for Dukes' C adenocarcinoma of the colon. This patient had a prolonged complete remission after intensive antileukemia chemotherapy.

The patient was a 68-year-old female who had a right hemicolectomy on April 21, 1977, for a Dukes' C adenocarcinoma of the cecum. She then received adjuvant chemotherapy with a total dose of 1170 mg of semustine and 29.25 g of 5-FU between June 13, 1977, and May 22, 1978. A followup examination in April 1980 showed a hemoglobin of 13.9 g/dl, a wbc count of 4800/mm^3, and a platelet count of 150,000/mm^3. Differential count showed 61% segmented neutrophils, 1% bands, 29% lymphocytes, 8% monocytes, and 1% eosinophils. She was admitted to the hospital on July 21, 1980, with a 1-day history of gum bleeding. Cbc on admission showed a hemoglobin of 7.7 g/dl, a wbc count of 3000/mm^3, and a platelet count of 28,500/mm^3. Differential count showed 9% segmented neutrophils, 6% bands, 58% lymphocytes, 2% monocytes, 3% eosinophils, 10% blast cells, and 9% metamyelocytes. Her only medication had been hydrochlorothiazide for hypertension. A bone marrow examination the day after admission demonstrated 90% cellularity, with 50% of nucleated cells being blast cells; a diagnosis of acute myelomonocytic leukemia (FAB M-4) was made. Chromosome studies demonstrated that chromosomes 3, 5, 6, 17, and 21 were missing, and there was an extra C chromosome similar to 22. She was treated with doxorubicin, vincristine, prednisone, and a 7-day infusion of cytarabine (Southwest Oncology Group protocol) on July 28, 1980. Repeat marrow examinations on Days 7 and 9 of treatment showed a persistent cellularity of 90%, and the patient received cytarabine for 5 more days.

The patient received another course of induction chemotherapy, including 7 days of cytarabine, on September 13, 1980, after a marrow examination had demonstrated 10% myelomonoblasts. Subsequent marrow examinations demonstrated a complete remission of her leukemia; a cbc on January 30, 1981, showed a wbc count of 8700/mm^3 with a normal differential count, hemoglobin of 12.9 g/dl, and a platelet count of 85,000/mm^3. After 12 months in complete remission and while receiving late intensification therapy with POMP (6-mercaptopurine, vincristine, methotrexate, and prednisone), the patient relapsed. She subsequently died during the reinduction chemotherapy.

DISCUSSION

In a recent bulletin to physicians, the National Cancer Institute reported on two cases of acute leukemia, two cases of smoldering leukemia, and one case of aplastic anemia arising approximately 1 year after completion of adjuvant semustine therapy in the Gastrointestinal Study Group protocol No. 6175 for colorectal carcinoma. There were a total of 280 patients treated in this protocol, with a median followup of 34–35 months. In the control arm of the protocol, no cases of leukemia were reported.

Other reports of acute leukemia after nitrosourea therapy include the development of acute myelocytic leukemia in a 69-year-old male who was treated over a 28-month period with only 67 g of streptozocin for metastatic islet cell carcinoma (1). The patient was observed to have progressive leukopenia which terminated in acute leukemia 13 months after the streptozocin therapy was stopped.

Cohen et al (2) reported two cases of acute nonlymphocytic leukemia associated with nitrosourea chemotherapy. The first case was a 56-year-old female who was treated for angioblastic meningioma initially with a total of 720 mg/m^2 of carmustine (BCNU), subsequently with 700 mg/m^2 of lomustine (CCNU), and finally with 10,000 mg/m^2 of teniposide (VM-26) between January 1970 and October 1973. She developed acute myelomonocytic leukemia in December 1974, which was preceded by several months of progressive leukopenia. The second case was a 60-year-old female who received a total of 2700 mg/m^2 of semustine for a left frontal oligodendroglioma. No other chemotherapy was given.

[1]Supported in part by Public Health Service grants CA-17984 and CA-15655 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

She developed progressive leukopenia during semustine therapy. This therapy was stopped, and 10 months later acute myelomonocytic leukemia was diagnosed.

In summary, therapy with nitrosoureas, especially if prolonged, has been associated with the development of acute leukemia. The leukemia may develop about 1 year after therapy has been discontinued and very frequently is preceded by a myelosuppressive phase. Survival after leukemia transformation has been notably very short, except in the case reported here. Of interest is the observation of a much higher risk of chemotherapy-related leukemia in patients > 40 years old compared to those < 40 (C. Coltman, personal communication).

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Experience of the Eastern Cooperative Oncology Group With Doxorubicin as a Single Agent in Patients With Previously Untreated Breast Cancer[1]

Various doxorubicin-containing combinations have been investigated over the past decade as alternatives to CMF (cyclophosphamide, methotrexate, and 5-FU) and, more recently, CMFP (CMF plus prednisone) combination chemotherapy for advanced breast cancer. Most of these trials involved the combination of doxorubicin and vincristine with or without other agents. However, a cohort of 19 patients initially treated with doxorubicin alone have not previously been reported, although brief reference to them has been made (1).

Table 1.—Patient characteristics

Characteristic	No. of patients
Performance status	
0–1	12
2–3	7
No. of sites involved	
1	2
2	9
≥ 3	8
Dominant site	
Soft tissue	1
Osseous	6
Visceral	12
Tumor-free interval (yrs)	
< 2	15
2–5	3
> 5	1

In 1973, the Eastern Cooperative Oncology Group (ECOG) initiated a trial of doxorubicin at a dose of 60 mg/m^2 given iv every 3 weeks to patients with advanced breast cancer not previously treated with chemotherapy. Standard ECOG criteria for treatment modification based on hematologic toxicity and for assessment of response were used. Ages of patients ranged from 37 to 75 years (median, 54). Patient characteristics shown in table 1 demonstrate a predominance of visceral involvement, short tumor-free intervals, multiple site involvement, and some limitation in performance status.

Eight patients (42%) responded: two with complete responses and six with partial (> 50% reduction in tumor diameter) responses. Four patients (21%) received only one course of therapy and were considered to have disease progression: one patient died 25 days after the first dose from unknown cause prior to reevaluation, one adrenalectomized patient died 10 days after the first dose from adrenal insufficiency precipitated by doxorubicin-induced vomiting, and two patients refused to continue treatment after one course (one due to doxorubicin-induced tachyarrhythmias).

The durations of response were 43, 63, 71, 139, 216, and 307 days for partial responses and 192 and 280 days for complete responses. Thus, the median duration of response was 5.5 months.

[1]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Participating institutions include: Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); Sydney Farber Cancer Institute, Boston, MA (CA-23318); Albert Einstein College of Medicine, Bronx, NY (CA-14958); University of Alberta, Edmonton, Canada; American Oncologic Hospital, Philadelphia, PA (CA-18281); Brown University, Providence, RI (CA-15947); Hahnemann Medical College, Philadelphia, PA (CA-13611); Jackson Memorial Hospital, Miami, FL; University of Florida-Jacksonville (CA-19818); Jefferson Medical College, Philadelphia, PA (CA-14215); University of Manitoba, Winnipeg, Canada; Mount Sinai Medical Center, New York, NY (CA-17152); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Rochester Cancer Center, NY (CA-11083); Tufts University, Walpole, MA (CA-07190); and Medical College of Virginia, Richmond (CA-10572).

Hoogstraten and Fabian (2) have recently reviewed the reported activity of single agents in the treatment of breast cancer. The composite response rate to doxorubicin was 40% (49 of 126 patients) for patients not previously treated with cytotoxic chemotherapy. The reported duration of response has been generally shorter than that of more recently studied drug combinations. The response rate of 42% for a duration of 5.5 months in the 19 patients reported here is consistent with that observation. It is noteworthy that in the next generation of studies, the combination of doxorubicin and vincristine produced a slightly higher response rate of 56% for a duration of 7.7 months in a comparable group of 166 patients not previously treated with chemotherapy (3).

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Pharmacology and Toxicity of Ip Streptozocin in Ovarian Cancer: A Case Report[1]

Streptozocin, composed of the cytotoxic moiety 1-methyl-1-nitrosourea attached to the carbon-2 position of glucose, produces diabetes mellitus in mice secondary to the destruction of pancreatic beta cells. Concentration of streptozocin in the beta cells of the mouse pancreas has been demonstrated, suggesting that the glucose carrier of streptozocin facilitates uptake into the islet cells (1). Streptozocin has demonstrated clinical antitumor activity against islet cell carcinomas (2). However, the mechanism of transport of streptozocin has not been directly investigated.

In this preliminary report we describe the toxicity and pharmacokinetics of ip administration of streptozocin to a patient with advanced ovarian carcinoma. The patient's tumor was rapidly consuming glucose, resulting in a peritoneal fluid glucose < 10 mg/100 ml.

Following the ip administration of 1 g of streptozocin, peripheral blood and peritoneal samples were obtained at various time points up to 5 hours. The EDTA-anticoagulated samples were immediately spun at 4000 rpms for 90 seconds to obtain plasma. One milliliter of plasma or peritoneal fluid was added to 0.25 ml of 3 N perchloric acid and kept at 0°C. The samples were then spun at 2700 × *g* for 10 minutes at 4°C. The level of streptozocin in the supernatants was determined by the method of Forist (3).

Prior to the administration of streptozocin, 100 ml of peritoneal fluid was removed, and cell-free fluid was obtained by centrifuging at 1000 × *g* for 10 minutes at 22°C. The half-life of streptozocin in vitro was determined by incubating streptozocin at a concentration of 300 μg/ml in the patient's peritoneal fluid with or without cells at 37°C.

CASE REPORT

A 54-year-old female presented with stage II moderately differentiated ovarian serous cystadenocarcinoma completely resected. Following surgery, the patient was given adjuvant chemotherapy with cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone. Six months after her initial surgery, she developed an abdominal mass and tense ascites, which at a second laparotomy proved to be unresectable recurrent ovarian carcinoma. She was then treated with hexamethylmelamine, cyclophosphamide, doxorubicin, and cisplatin. She improved during this therapy but never obtained a partial response. Within 5 months she demonstrated progressive disease, with an increase in the number of paracenteses required and a declining performance status. She was then treated ip with doxorubicin and cisplatin without any evidence of response.

The patient's peritoneal glucose was always < 10 mg/100 ml (except transient elevations following the ip doxorubicin and cisplatin). Cultures for tuberculosis, fungus, and bacteria were always negative, and cytology was positive. Since it appeared that the tumor was avidly consuming glucose, a course of ip streptozocin was given. The patient received 1 g of streptozocin ip six times within a 3-week period. There was no nausea, vomiting, or evidence of renal dysfunction following this chemotherapy.

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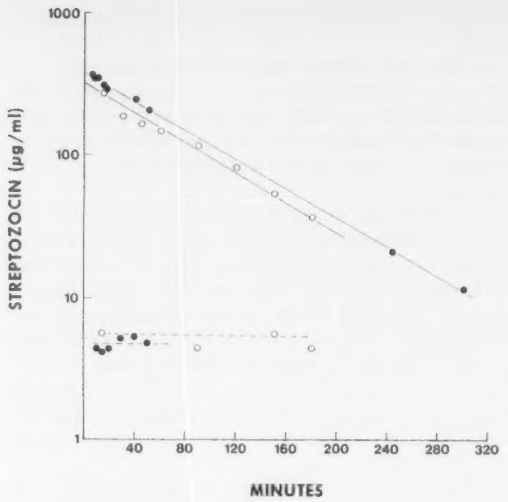

FIGURE 1.—Plasma (broken lines) and peritoneal (solid lines) streptozocin levels following the initial ip administration of 1 g of streptozocin (closed circles) and the 2nd administration 5 days later (open circles).

Serial serum and peritoneal amylases were normal. Streptozocin levels in the plasma and peritoneal fluid were measured on two occasions. The patient's disease continued to progress, and she died. Permission for an autopsy was not obtained.

Peritoneal and plasma streptozocin levels were determined as described (fig 1). There was minimal leakage of streptozocin into the systemic circulation. The half-life of streptozocin in the peritoneal fluid was 61 ± 0.5 minutes (r correlation > 0.995), with a volume of distribution of 3 ± 0.4 liters. The half-life of streptozocin in the patient's peritoneal fluid with or without cells in vitro at 37°C was 42 ± 10.4 minutes. The in vitro half-life of streptozocin was shorter than the in vivo half-life.

DISCUSSION

The patient tolerated several ip doses of streptozocin without nausea, vomiting, or evidence of renal dysfunction. The patient did not respond to the therapy, but dose-limiting toxicity was not reached. The pharmacokinetic studies demonstrate that streptozocin was largely confined to the intraperitoneal cavity. The volume of distribution (3 liters) is approximately equivalent to the volume of ascitic fluid present. The shorter in vitro half-life of streptozocin as compared to the in vivo intraperitoneal half-life also suggests that there was no significant absorption into the systemic circulation, since significant absorption into the systemic circulation should have reduced the in vivo intraperitoneal half-life.

Ovarian carcinoma is often limited to the abdomen. In this preliminary report, streptozocin administered ip did not have any significant toxicity and remained largely intraperitoneal. Perhaps the chloroethylnitrosoureas (eg, carmustine) may share the same pharmacokinetics as streptozocin, ie, remain largely intraperitoneal following ip administration. Since all the nitrosoureas have a very short half-life in serum (usually ≤ 60 minutes), they may prove particularly useful for ip therapy, resulting in a very high local concentration without significant systemic toxicity (4). Further studies with nitrosoureas administered ip to patients with malignancies confined to the peritoneum will be necessary to determine if this approach will be useful.

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Trial of AMSA in Acute Leukemia[1,2]

The acridine derivative AMSA has had antitumor activity in preclinical systems and has demonstrated

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[2]Presented at the 18th Annual Meeting of the American Society of Clinical Oncology, April 30–May 2, 1981.

antileukemic activity in humans (1). The latter has been achieved with total drug doses > 360 mg/m² delivered in 3–5 days (2–6). We wish to comment on a trial done at the National Academy of Medicine in Buenos Aires, Argentina, that adds to the present experience with AMSA, especially in lymphoblastic leukemia. In a recent study, we administered AMSA to 17 patients with heavily pretreated acute leukemia. There were ten patients with lymphoblastic leukemia (ALL), six with myelogenous leukemia (AML), and one with accelerated (blast) phase chronic myelogenous leukemia (BC-CML). All had been pretreated with daunorubicin. The AMSA dose was 75–90 mg/m²/day iv × 7. The median age of the patients with ALL was 15 years (range, 6–36), and eight of them were between 10 and 26 years old. Of these ten patients with ALL, two had bone marrow responses to AMSA after one drug course: one achieved a complete response (CR) lasting 49 days and the other achieved a partial response (PR) lasting 21 days. Both patients received a second course but had no further improvement. A third patient had a 70% reduction in spleen size after two courses but no change in bone marrow. Four of the remaining patients achieved aplasia but showed only return of leukemia cells. All patients showed transient reduction or clearing of circulating blast cells from peripheral blood. Among the other six patients, one with AML had a complete bone marrow response lasting > 280 days and the others showed only transient reduction in circulating blast cells.

The toxic effects of AMSA included nausea and vomiting, alopecia, local pain, phlebitis, and transient skin rash. Of particular note was the occurrence of jaundice in four patients. One 10-year-old girl developed liver failure and died in hepatic coma 2 weeks after the first AMSA cycle was completed. The serum transaminases were repeatedly normal, but the total bilirubin rose to 21 mg/dl prior to death. She had a positive hepatitis B antigen titer but no history of overt hepatitis. She was not taking excessive doses of acetaminophen or other drugs.

These results show clinical activity of AMSA in ALL and compare favorably with results from other centers. Legha et al (2) achieved 13 CRs and six PRs in 56 patients, but none of these occurred in 13 adult patients with ALL. Arlin et al (3) achieved three CRs in ten patients with ALL and in three of 17 with acute nonlymphocytic leukemia (ANLL), but achieved none in four patients with BC-CML.

Van Echo et al (4) achieved four CRs in 17 patients with ANLL and one PR in five with ALL, but no response in five with BC-CML. Land et al (5) achieved six CRs and three PRs in 35 children with leukemia, including three CRs and two PRs in 21 patients with ALL. One additional report (6) showed two CRs in nine children with advanced lymphoblastic leukemia.

Our data combined with those in the other reports indicate a total of 13 observed CRs and PRs in 68 patients of all ages with ALL. The drug deserves further trial as part of induction and consolidation regimens for lymphoblastic leukemia. However, the potential of AMSA to produce jaundice and liver dysfunction needs emphasis, and patients with preexisting liver disease or who are receiving other hepatotoxic drugs such as acetaminophen should be watched carefully while receiving AMSA therapy.

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Approaches to the Management of Pain

Oakland, California, September 9, 1982

This 1-day conference will deal with the multidisciplinary approach to the management of pain. Cosponsoring organizations are the Bay Area Tumor Institute and the East Bay Cancer Program. The program will be held in Goodman's Hall, 10 Jack London Square.

For further information, contact: Despina C. M. Johnson, MPH, Program Manager, 2844 Summit St, Suite 204, Oakland, CA 94609 (telephone: 415-465-8570).

Urologic Cancer Course

Boston, Massachusetts, September 22–24, 1982

This intensive 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. The course will emphasize the multidisciplinary approach that constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. Although teaching will be primarily provided through lectures, panel discussions addressing major current controversies in urologic cancer will be an integral portion of the course. A comprehensive syllabus will be provided.

Guest faculty will include Drs. Lawrence H. Einhorn, Donald Skinner, Willet F. Whitmore, Jr, and Leonard Zinman. The Harvard faculty participants will include leaders in urologic cancer from the Sidney Farber Cancer Institute, Brigham & Women's Hospital, Joint Center for Radiation Therapy, Children's Hospital Medical Center, Beth Israel Hospital, and Massachusetts General Hospital.

The course fee is $325.00.

For further information, contact: Harvard Medical School, Department of Continuing Education, Boston, MA 02115 (617-732-1525).

Paraneoplasia: Basic Concepts and Clinical Aspects

Brussels, Belgium, November 5–6, 1982

This 2-day symposium presented by the European Organization for Research on Treatment of Cancer will focus on endocrine paraneoplasia, neurologic paraneoplasia, coagulopathies, and nonhormonal tumor markers. The Chairmen are J. Waldenström and H. J. Tagnon.

In addition, free communications of 10 minutes each will be accepted. An abstract must be sent to the address below before September 30, 1982. Seating is limited and preregistration is recommended.

For further information, contact: Dr. M. Staquet, Director, EORTC Data Center, rue Héger-Bordet 1, 1000 Brussels, Belgium. Telephone: (2) 538.57.90, Telex: 22773.

Gastrointestinal Tumor Study Group

The Gastrointestinal Tumor Study Group (GITSG), an NCI-funded, multidisciplinary clinical trials group, invites institutions and individuals who are forming a Community Clinical Oncology Program (CCOP) to participate in GITSG colon, rectal, gastric, and pancreatic studies. *For information, please contact* Philip S. Schein, MD, c/o ECTO Operations Office, Suite 214, 11325 Seven Locks Rd, Potomac, MD 20854. Telephone: 301/299-8655.

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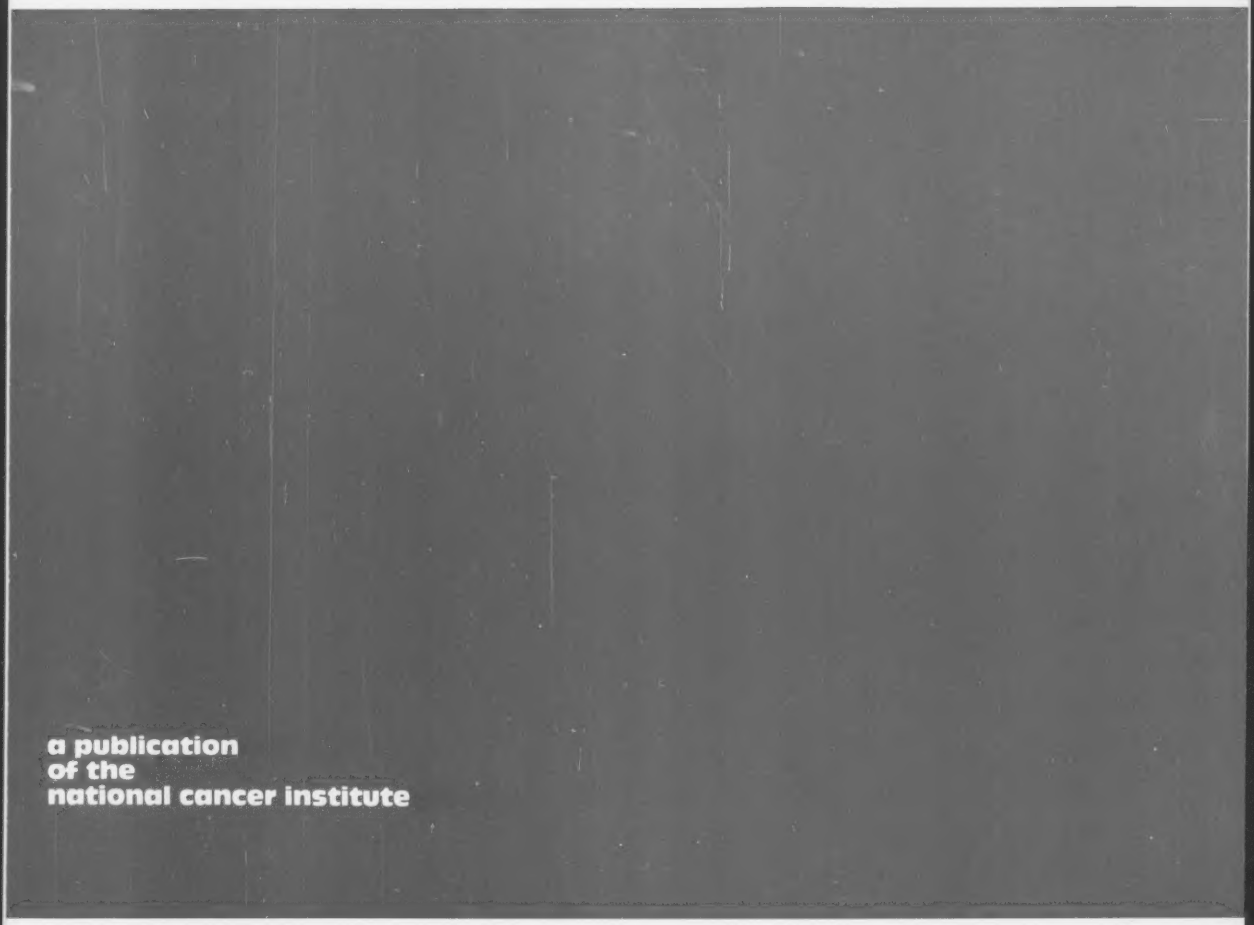

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